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A COMPARISON OF THE FUNCTION OF THE GLOMERULAR AND AGLOMERULAR KIDNEY[1]

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Received for publication January 23, 1930

Practically all investigations attempting to unravel the processes involved in the function of the kidney have been made under conditions of anesthesia and operative interference. This has been necessarily so because the main problem involved, the assigning of functions to the different parts of the renal unit and the discovery of the nature of the processes involved in these functions, has seemed incapable of approach except under simplified conditions where variables could be controlled. Perfusion of the isolated organs represents an extreme case of the artificiality of the conditions of the kidney in the experiment compared to those in a normal animal. Just how far it is justified to transfer conclusions obtained in experiments where the conditions are abnormal to the normal is not known in this case: it is certain, however, that in many instances the wrong conclusion about the functioning of an organ in the intact healthy animal has been made when the experiments giving this conclusion were done on animals subjected to narcosis and operative interference.

A second method of approach to a study of the function of the kidney has been a comparison of the changes in the blood plasma and urine under different conditions, a method which can be applied to the normal unanesthetized animal or man. This second method, unfortunately, has not told us much about the main problems involved in kidney function, the nature of the processes occurring in the morphologically different parts of the renal tubule. Although we have here perfectly normal conditions, the process

[1] These results were presented at the XIIIth International Physiological Congress, Boston (This Journal, 1929, xc, 446).

[2] A few experiments on the goosefish were carried out at the Marine Biological Laboratory, Woods Hole, in the summer of 1928. I wish to thank Mr. Allan L. Grafflin for assistance in these experiments at Woods Hole.

of urine secretion is too complex and the variables too many to allow an adequate attack on the above problem.

Still a third method of approach, one which seems to combine the advantages of the two former, is possible. This is a comparative study of morphology and function of the renal unit as it has been gradually evolved from a simpler to a more complex structure. A survey of what is known of the kidneys of vertebrates indicates at once that striking morphological differences exist among the different classes of animals. If these morphological differences can be correlated with distinct differences in function, one should be able to assign functions to many parts of the renal tubule. Although this method has not been employed to any extent in the past, it should prove particularly valuable in eliminating the disturbing effects of anesthesia and operative procedures: it can be used on animals normal in every way by allowing nature to carry out the experimental modification of the renal unit. The present report utilizes this method by comparing the function of glomerular and aglomerular kidneys.

In previous communications (Marshall and Grafflin, 1928; Marshall, 1929), studies on the morphology and to some extent the function of the aglomerular kidneys of the goosefish (*Lophius piscatorius*) and the toadfish (*Opsanus tau*) have been reported. The kidney of the toadfish does not possess Malpighian corpuscles, and the few which occur in the kidney of the goosefish have now been conclusively shown to be non-functional (Grafflin, 1929). For a comparison between the glomerular and aglomerular kidney, it would be best to compare two fish in which the renal units differed only in the presence or absence of a glomerulus, the tubular part being identical in both. Unfortunately, at present, our knowledge of the epithelium of the tubule of different fish kidneys is not sufficient to allow us of being certain of doing this. Thus, in the aglomerular kidney of the Lophobranchs and *Lophius*, Verne (1921–22) and Edwards (1928) state that the tubule contains two types of epithelium (with and without brush border) exclusive of the collecting tubule, while Audigé (1910) describes only one type for both the glomerular and aglomerular (*Lophius*) tubule of teleosts. Edwards (1929) has recently described the tubule of certain marine teleosts as containing an epithelium with brush border throughout and believes that this brush border segment can be subdivided into distinct parts. On the other hand, Policard and Mawas (1906) find two types of epithelium (with and without brush border) in the glomerular kidney of certain teleosts. In sections of the kidney of the toadfish we have been unable to find more than one type of epithelium, although in those made from the glomerular kidneys of certain teleosts the two types described by Policard and Mawas are easily identified. It is quite probable, therefore, that both glomerular and aglomerular tubules of teleosts are of two types, although more work must be done before the matter can be definitely settled. Comparison of

the function of the glomerular and aglomerular kidney will at present permit us, therefore, only to determine 1, what functions an aglomerular tubule can carry out; 2, what additional functions can be performed when a glomerulus is added at the beginning of the tubule. In other words, such a study indicates clearly what functions the glomerulus possesses which cannot be delegated to the tubule alone.

METHODS. The goosefish and toadfish have been studied as aglomerular kidneys, while the sculpin, flounder, carp, and eel have served as representatives of marine and fresh water fish with glomerular kidneys. The species employed are given in table 3. The experiments on the goosefish, sculpin, and flounder were done at Salisbury Cove, Maine, while those on the toadfish, carp and eel were carried out in the laboratory at Baltimore. Blood and urine were collected from the goosefish as previously described (Marshall and Grafflin, 1928). In the other fish, the bladder was emptied by a fine glass catheter, a ligature secured around the urinary papilla, and after a certain time interval removed and the bladder again emptied. The bladders of all these fish are capable of holding several cubic centimeters of urine and were never completely distended when emptied. On account of the large urine flow of fresh water fish, it was found more convenient in the case of the eel and carp to tie in a glass catheter attached to a rubber bag and allow the urine to collect in the latter.

The toadfish were kept in dilute artificial sea water (225 MM. chloride per liter), the eel and carp in fresh water, and the other marine teleosts in aquaria containing running sea water (500 MM. chloride per liter).

Chlorides were estimated by the Van Slyke (1923) method, and glucose by the Folin-Wu (1920) or Hagedorn-Jensen (1923) methods.

RESULTS. *Urine flow.* As regards the relative amounts of urine eliminated by the glomerular and aglomerular kidney, it is difficult at present to make a definite statement. The conditions under which urine flows have been measured could not always be made comparable and widely different rates have been obtained for the same species of fish. There is reason to believe, however, that the higher rates of urine flow obtained on marine teleosts are not the true ones under normal conditions of existence. Thus, the urine of the goosefish, as obtained under experimental conditions, is low in total nitrogen and high in chloride, while specimens of urine obtained by Grollman (1929) off St. George's Banks in the Atlantic were high in total nitrogen and low in chloride, which can be interpreted as meaning a much lower urine flow; a short period of asphyxia can raise the urine flow of the sculpin from 6 to 86 cc. per kilo per day with a marked rise in chloride concentration; and frequently a very high urine flow in the sculpin and the toadfish has been followed by the death of the animal. Such figures as we have are as follows: wrymouth (8 specimens), 1.5 to 11.0 cc.; sculpin (16 specimens), 3.2 to 57.0 cc.; goosefish (11 specimens), 12.9 to

54.4; toadfish (15 specimens), 0.6 to 9.4 cc. per kilo per day. The toadfish were kept in sea water containing 225 millimols of sodium chloride per liter, the other fish in full strength sea water containing about 500 millimols of sodium chloride per liter. From these data, one cannot conclude that there is any essential difference in the rate of urine flow of glomerular and aglomerular marine teleosts.

Composition of urine. No significant difference has been found in the character of the urine obtained from aglomerular as opposed to glomerular marine teleosts. The presence of large amounts of magnesium and sulphate, the occurrence of only small amounts of urea, and relatively large amounts of creatine and undetermined nitrogen are characteristic of the urines from both glomerular and aglomerular forms (Sulze, 1922; Marshall and Grafflin, 1928; Smith, 1929). In the urine of the toadfish, which has not been previously examined, the total nitrogen has been found to vary

TABLE 1

Chloride content in millimols per liter of plasma and urine of aglomerular fish

GOOSEFISH		TOADFISH	
Plasma	Urine	Plasma	Urine
168	121	118	72
171	72	120	34
174	75	126	80
188	114	131	0
193	196	138	0
200	219	141	0
228	229	144	137
		147	10
		150	94
		173	191

from 13 to 132 mgm. per cent, the urea-N from 2.1 to 8.4 mgm. per cent, and a large fraction of the total nitrogen to be unaccounted for by urea, uric acid, creatine, creatinine, ammonia or amino acids. In the case of the goosefish, Grollman (1929) has recently shown in this laboratory that the unidentified nitrogen in its urine is trimethylamine oxide. Whether this or other compounds account for the unknown nitrogen of other fish urines remains to be determined.

The presence of a threshold for certain substances present in plasma, below which they are not excreted, is a characteristic of the kidney of the frog and mammal. That such a threshold phenomenon for chloride exists in the case of the glomerular fish kidney is shown by examining urine from eels in salt and fresh water. In the former case the urine may contain large amounts of chloride, in the latter it rarely contains more than a trace.

The phosphate in the urine of the goosefish may vary from 0.1 to 18.3 millimols per liter, where the plasma contains 5 or 6 millimols (Marshall and Grafflin, 1928).

Table 1 indicates that the threshold phenomena for chloride as well as phosphate is exhibited by the aglomerular kidney. In the case of the toadfish, the chloride content of the external medium was varied.

From data on the freezing points of the blood plasma and urine to be found in the literature, it can be concluded that the urine of both glomerular and aglomerular marine teleosts is either isotonic or hypotonic to the blood plasma, never hypertonic. Apparently, it is only with the development of the loop of Henle in the bird that hypertonic urine can be secreted by the kidney.

Glycosuria. In mammals glycosuria can be produced either by a rise of the blood sugar above the threshold value or by a lowering of the renal threshold for glucose. The production of the hyperglycemia necessary to cause sugar to appear in the urine can be brought about in many ways, injection of glucose, administration of adrenalin, asphyxia, removal of the pancreas, etc. The one experimental method to produce a glycosuria due to a lowering of the renal threshold is the administration of phlorizin.

In the first experiments on fish qualitative tests for glucose were made on the urine with Benedict's solution. A positive test in the urine for glucose has been obtained after a rise of blood sugar in the sculpin, eel and smooth dog fish; and, after the injection of phlorizin (200 mgm. per kilo) in these fish and in the flounder. In fish with aglomerular kidneys a qualitative test for glucose has never been obtained by producing a hyperglycemia, by the injection of phlorizin, or by a combination of both of these methods. The following two summaries of experiments on the goosefish are typical of several which have been performed.

Experiment 1. Goosefish, ♀, 9.3 kilos. Injected intravenously 10 grams of glucose in 25 cc. of water. The blood plasma sugar was 512 mgm. per cent 7 minutes, 364 mgm. per cent 27 minutes, and 350 mgm. per cent 57 minutes after the injection. Urine samples obtained at 10, 30 and 60 minutes after injection were all found negative for glucose.

Experiment 2. Goosefish, ♂, 5.3 kilos. Injected 0.9 gram phlorizin and 8 grams of glucose. The blood sugar before injection was 18 mgm. per cent, and two hours after injection was 322 mgm. per cent. Six samples of urine obtained in the five hours following injection were all negative for glucose.

Further experiments were carried out with a quantitative determination of the glucose in urine as well as blood. In most of these reducing substances were determined on the urine without preliminary treatment; in a few experiments, however, a careful determination of the amount of glucose in the urine was made by the use of Lloyd's reagent and fermentation with yeast (Van Slyke and Hawkins, 1929).

Table 2 illustrates typical determinations of the reducing bodies in blood and urine of several marine teleosts before and after the injection or phlorizin (200 mgm. per kilo). The hyperglycemias were produced accidentally, probably by asphyxia and handling the fish (McCormick and MacLeod, 1925).

The following experiment on the goosefish is particularly instructive.

Experiment 3. Goosefish, ♂, 9.2 kilos. Sample of urine from bladder showed 46 mgm. per cent of reducing substance, none of which was found to be glucose (Lloyd's reagent and yeast fermentation). Ten grams of glucose and 1.8 grams of phlorizin were injected intravenously and urine collected quantitatively in four samples during the next 24 hours. These samples showed 46, 63, 69, and 30 mgm. per cent respectively reducing substance, while the last three gave 28, 34, and 9 mgm. per cent of glucose by fermentation. Blood taken 10 minutes after the injection contained 510 mgm. per cent of glucose and 2½ hours later, 160 mgm. per cent.

TABLE 2

Reducing substances in blood and urine

NAME	GLUCOSE, MGM. PER CENT IN		
	Urine before phlorizin	Urine after phlorizin	Blood
Sculpin	28	130	100
Sculpin	518	804	361
Flounder	46	196	70
Toadfish	44	66	360
Goosefish	44	49	

From these results, it can be concluded that glycosuria can be easily produced in fish with glomerular kidneys by hyperglycemia and by the administration of phlorizin; but that in the fish with aglomerular kidneys, it is impossible to find more than a mere trace of glucose in the urine after either of the above methods used alone or in combination.

Elimination of foreign substances. In addition to the ordinary constituents found in the blood, the kidney eliminates a great variety of foreign bodies which may enter the circulation. The mammalian kidney, which has been studied almost exclusively in this respect, excretes to some extent practically all substances which are soluble in water and readily diffusible. As a contrast to this widespread elimination of different foreign substances by the kidney, the specificity in the elimination of foreign substances exhibited by the digestive glands (salivary glands, pancreas, liver) may be mentioned. The experiments to be recorded would seem to show that this catholicity of the kidney is dependent on the presence of glomeruli: an aglomerular kidney exhibits specificity for foreign bodies like the true secretory gland.

Thus, it has been found that not all soluble and diffusible substances which are eliminated by the glomerular kidney are excreted by an aglomerular one. Phenol red and sodium ferrocyanide have been tested particularly in this study. These substances were injected intramuscularly in dosage of 4 mgm. per kilo for phenol red and 300 mgm. per kilo for sodium ferrocyanide, the urine being tested for the presence of the former by means of alkali and for the latter by the addition of hydrochloric acid and a ferric salt. The presence or absence of a blue or green color within a few minutes of adding the reagents was taken as a positive or negative reaction for ferrocyanide. The experiments on injection and excretion of both substances were repeated two or three times on each species of animal except in the

TABLE 3

Excretion of phenol red and ferrocyanide by vertebrate kidney

SCIENTIFIC NAME	COMMON NAME	PHENOL RED	FERROCYANIDE
Myxine glutinosa	hagfish	+	+
Raia erinacea	skate	+	+
Squalus acanthias	spiny dog-fish	+	+
Mustelus canis	smooth dog-fish	+	+
Myozocephalus octodecimspinosus	sculpin	+	+
Pseudopleuronectes americanus	flounder	+	+
Cryptacanthodes maculatus	wrymouth	+	+
Lophius piscatorius	goosefish	+	−
Opsanus tau	toadfish	+	−
Cyprinus carpio	carp	+	+
Anguilla rostrata	eel	+	+
Protopterus ethiopicus	lung fish		+
Necturus maculosus	mud puppy	+	+
Rana catesbiana	bull frog	+	+
Bufo americanus	toad	+	+
Malaclemmys palustris	slider terrapin	+	+
Gallus domestica	chicken	+	+

case of the fish with aglomerular kidneys where the failure of ferrocyanide to be eliminated in the urine was confirmed in many experiments, in some of which the substance was given by intravenous injection. Table 3 gives the results obtained on representative species from different classes of vertebrates. The urine of the hagfish was obtained by killing the animal some hours after the injection, opening the abdomen, and inserting a capillary glass tube into the lower end of the mesonephric duct. I have to thank Dr. H. W. Smith for performing, in Africa, the experiments on the lung fish.

It is well known that both phenol red and ferrocyanide are eliminated by the mammalian kidney. One can be certain, therefore, that the kid-

neys of all vertebrates eliminate phenol red, and that the elimination of ferrocyanide fails only in the case of animals with aglomerular kidneys (goosefish and toadfish). That this lack of elimination of ferrocyanide by the aglomerular kidney is not due to rapid removal of the substance from the blood stream or to combination with the proteins has been proven by finding in the blood plasma at the end of the experiment, large amounts of ferrocyanide which can be ultrafiltered through collodion.

In practically all the above experiments the bile present in the gall bladder has been tested for the presence of phenol red. The result obtained in a qualitative way has shown that in the lower vertebrates much more phenol red is eliminated by the liver than by the kidney and as we ascend the evolutionary scale more and more is eliminated by the kidney, so that in mammals we find only a trace at most in the bile. It is interesting to note, however, that in mammals with damaged kidneys, large amounts can be found in the bile (Underhill and Blatherwick, 1914).

In the case of the elimination of glucose, it has been shown that the impermeability of the tubule is only a relative matter: with very high blood sugar and injection of phlorizin there appear in the urine traces of glucose comparable to the trace normally present in human urine, which is ordinarily considered to be free of sugar. That the tubule should be completely impermeable to ferrocyanide was thought unlikely; and, indeed, it was soon found that the application of a more delicate test to the urine after ferrocyanide injection reveals traces of ferrocyanide. Thus, if after the addition of hydrochloric acid and ferric ammonium citrate to the urine, the colorless solution was allowed to stand for an hour or more a very light greenish-blue color developed. The difference in the elimination of ferrocyanide by the two types of fish kidney is very remarkable. Thus, the sculpin eliminated about 2.5 to 5.0 per cent of the injected ferrocyanide in 5 hours, while in the same time a goosefish eliminated only 0.013 per cent. This means that the glomerular marine fish kidney is about 300 times as efficient in eliminating ferrocyanide as the aglomerular.

It is to be noted that we have taken phenol red and ferrocyanide as representatives of two types of foreign bodies, one excreted and one not by the aglomerular kidney. This indicates the specificity of the tubule in eliminating foreign substances, but by no means should be taken to mean that other secretory glands will act in the same way to the two substances used. Indeed we know that neither ferrocyanide nor phenol red is eliminated by the salivary gland, whereas both are readily excreted by the liver of the lower vertebrates.

Discussion. The present study proves unquestionably that the renal tubule can be excretory as well as reabsorptive in its function: that it can pass substances from the blood and lymph across the tubule into its lumen. Moreover, it appears that the tubule alone in the aglomerular

kidney can carry out most of the functions which are performed by the tubule and glomerulus in the glomerular fish kidney. The urine secreted by the aglomerular and glomerular kidney is not essentially different in its composition; certain substances (chloride and phosphates) which may be present in the urine from the glomerular kidney in much smaller concentration than in the blood plasma are also found to be retained by the aglomerular kidney; while, on the other hand, the aglomerular kidney differs functionally from the glomerular one in its inability to excrete glucose and in its selective behavior in eliminating foreign bodies.

The failure or relative failure of the tubule to excrete glucose when the blood sugar is very high and when the animal is phlorizinised shows that filtration across the tubule at one level and reabsorption lower down cannot explain the excretory function of the aglomerular kidney. It must be concluded, then, that the aglomerular kidney is secreting in the sense that the tubule is removing substances at low concentration from the blood and transferring them at higher concentration into its lumen. As to the nature of this excretory process of the tubule, there is no information. It would seem, however, that the process in the tubule can be compared to that of the secretion and concentration of bile pigments and dyes by the liver.

It is reasonable to assume that the presence of a glomerulus at the beginning of the tubule of fish has not completely abolished its excretory function. In marine teleosts with glomerular kidneys, glucose is concentrated by the kidney only 2 to 3 times as a maximum when phlorizin has been administered, while sulphate, magnesium, and phenol red may be concentrated 30 times or even more. Since these substances (sulphate, magnesium and phenol red) are known to be eliminated and concentrated by the aglomerular tubule, the obvious inference would appear to be that they are eliminated also in part by the tubule of the glomerular fish kidney.

SUMMARY

A comparison of certain functions of aglomerular as opposed to glomerular kidneys of fish has been made. The composition of the urine of marine teleosts is found to be essentially the same in both types of fish. The threshold phenomena shown by the glomerular kidney is exhibited by the purely tubular kidney: chloride and phosphate may disappear from the urine under some conditions and under other may be present in large amounts. The aglomerular kidney differs from the glomerular one in showing a specificity in the elimination of foreign bodies. Thus, the aglomerular kidney eliminates phenol red, but fails to eliminate the highly diffusible ferrocyanide. Glycosuria is easily produced in fish with glomerular kidneys, but only a trace of glucose ever appears in the urine from an aglomerular kidney, even when the blood sugar is high and phlorizin has been given.

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GLYCOGEN FORMATION IN RATS

I. Diets Containing about Sixty Per Cent of the Total Caloric Value in the Form of Starch, Sucrose, Lard and Casein

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Studies on liver and muscle glycogen in rats have been made by Karczag (1), Macleod (2), Foster (3), Cori (4), Bodey (5), and Barbour (6).

We have used thirty litters of Long and Evans rats. Each litter was separated from the mother on the twenty-second day, and one rat placed on each of the diets. In addition to having as much food and water as desired, each rat received three drops of cod liver oil and one-fourth teaspoonful of equal parts of yeast and wheat germ, daily. After about three

TABLE 1

DIET	LIVER GLYCOGEN	Diff. / P.E.	LIVER GLYCOGEN PER GRAM BODY WEIGHT	Diff. / P.E.	MUSCLE GLYCOGEN	Diff. / P.E.
	per cent		*mgm.*		*per cent*	
1—Starch, 33 cases	3.736±0.11		1.803±0.062		0.3096±0.0110	
Difference	+0.398±0.15	2.6	+0.112±0.086	1.3	+0.0125±0.0140	1.0
2—Sucrose, 30 cases	4.434±0.12		2.394±0.080		0.2941±0.0095	
Difference	+1.096±0.16	6.8	+0.703±0.100	7.0	−0.0030±0.0120	1.0
3—Lard, 30 cases	3.854±0.10		1.913±0.055		0.3139±0.0092	
Difference	+0.516±0.15	3.4	+0.222±0.081	2.7	+0.0168±0.0120	1.4
4—Casein, 30 cases	2.681±0.10		1.445±0.063		0.3344±0.0140	
Difference	−0.657±0.15	4.4	−0.246±0.087	2.8	+0.0373±0.0160	2.3
5—Balanced, 30 cases	3.338±0.11		1.691±0.060		0.2971±0.0081	

weeks (18–22 days) on the special diets, the rats being now six weeks old, the liver and muscle glycogen contents were determined, after a two-hour starvation period.

The composition of the diets used was given in our preliminary report (7), as was the method of killing and analysis.

The minimum daily gain acceptable in the males was 2.8 grams; in the

females, it was 2.0 grams. Since no sexual difference was evident in the liver and muscle glycogen contents on any one of the test diets, we shall not give the results separately for the two sexes.

Table 1 presents the results for each diet, together with the differences between each test diet and the balanced diet, using the latter always as the subtrahend.

Feeding 60 per cent of the total calories in the form of cornstarch gave a liver glycogen which did not differ significantly from that on the balanced diet. With 60 per cent of the caloric value in the form of either sucrose or lard, a significantly higher liver glycogen was found, while 60 per cent of casein gave a significant decrease. The muscle glycogen content on any one of the test diets did not differ significantly from that on the balanced diet.

We desire to express our gratitude to Dr. C. M. Jackson and to Dr. G. O. Burr for the criticism received.

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THE RÔLE OF THE CENTRAL NERVOUS SYSTEM IN THE CONTROL OF PANCREATIC SECRETION

I. The Secretion of Insulin during Hyperglycemia

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Numerous workers since the pioneer investigation of von Brazol in 1884 (1) have confirmed his finding that the administration of glucose is followed by an hypoglycemia. By the method of pancreatic-jugulars-anastomosis Zunz and La Barre (2) established the fact that the secondary hypoglycemia is chiefly due to hypersecretion of insulin and that the stimulation of the pancreas is of parasympathetic origin. Since this stimulation arises in the cephalic division of the central nervous system, the present work was undertaken to determine the approximate position of glycosensitive centers controlling peripheral insulin-secretion particularly during hyperglycemia. To settle this question, we used a combination of J. F. and C. Heymans' isolated-head technique with the method of pancreatic-jugular anastomosis.

Technique. As can be seen in figure 1, the complete technique involves the use of three dogs. The head of dog B is kept alive by a double cross-circulation between B's head and the trunk of dog A. The head of B is isolated completely from its trunk except for the two pneumogastric nerves. There is then made an anastomosis between the pancreatic vein of dog B and the jugular vein of dog C. Dog C is the test-object for hypernormal insulin-secretion by dog B.

With such preparations, we performed two series of experiments. In the first series, before establishing a double cross-circulation between dogs A and B, we removed the cerebral hemispheres of the isolated head of B. This operation was carried out by the following method: After a large fronto-occipital incision, one exposes the parietal and temporal regions on both sides. By an extended bilateral bone excision, one obtains an exposure of the hemispheres. After incising the dura mater, one removes successively with a blunt instrument, the occipital, parietal, temporal and frontal lobes. The corpus callosum is generally injured during this procedure; but the subjacent thalamic region always remains intact. If one is careful not to produce any injury of the sagittal and transverse sinuses, a slight hemorrhage is produced only when tension is applied to the cerebral

peduncles. This can be quickly stopped by the gentle application of a gauze-tampon.

In a second series of experiments, before establishing the transfusion of the isolated head, we removed not only the cerebral hemispheres but also the thalamic region. For this operation one uses the technique just described and makes a frontal section at the level of the groove which separates the thalamus from the pineal gland. The hypothalamic region including the hypophysis, tuber cinereum and corpora mamillaria, is not eliminated by this section. However it is quite possible that by too vigorous application of tampons the circulation may be interfered with in these hypothalamic centers.

Fig. 1. A. Chloralosed dog the carotid arteries and jugular veins of which are anastomosed with the similar vessels of dog B.

B. Chloralosed dog the head of which is completely separated from its trunk except for the two vagi.

C. Chloralosed dog the jugular vein of which is anastomosed with the principal pancreatic vein of dog B.

Usually then we performed an experiment as follows: One takes three dogs, A, B, and C which are chloralosed, and isolates the carotid arteries, jugular veins and vagus nerves in the necks of dogs A and B. After setting the chains of the Chassaignac's breakers in the lower and upper parts of the neck, one begins artificial respiration of dog B. This dog is then placed ventral side down for the opening of the parietal and temporal regions. After the removal of the hemispheres with or without frontal section and after the tamponing of the cerebral cavity, the animal is replaced in a dorsal position as near as possible to dog A and the cross circulations between the carotids and carotids, and jugulars and jugulars of dogs A and B, and between the principal pancreatic vein of dog B and a jugular of dog C are established. There are now injected 60 cc. of a 20 per cent dextrose solution into the saphenous vein of dog A. After 20 to 30 minutes of pancreatic-jugular transfusion, one separates dogs B and C. Samples of blood are taken from the femoral arteries of the three dogs before and immediately after interruption of the pancreatic transfusion. Moreover samples of blood are removed hourly from dog C (test-object of insulinemia) after the transfusion has been stopped. The blood-sugar concentrations of these different samples are determined by the method of Hagedorn and Jensen.

EXPERIMENTAL RESULTS. *A. Response to hyperglycemia by animals without cerebral hemispheres.* Using the technique previously described, we showed that the increase of insulin-secretion consequent to hypergly-

cemia was due to a central stimulation. In the present experiments we have tried to determine whether or not a sudden increase in the blood-sugar level produces hyperinsulinemia in animals without cerebral hemispheres. In table 1 may be found the data obtained in four experiments performed under these conditions.

It is clear that the dextrose-concentration of the femoral blood of the three dogs does not vary appreciably before the beginning of the transfusion and before the injection of dextrose into dog A. At the time the pancreatic-jugular transfusion is stopped, the blood of dog A is markedly hyperglycemic consequent to the dextrose injection. The blood-sugar concentration of B does not change notably. But the blood-sugar level of dog C becomes distinctly low up to two hours after the transfusion; then the blood-sugar rises progressively to the normal level. These results

TABLE 1

The response to hyperglycemia after the removal of the cerebral hemispheres

DOG	FEMORAL BLOOD REMOVED	DEXTROSE IN GRAMS PER LITER OF BLOOD IN EXPERIMENT			
		I	II	III	IV
A	Before dextrose injection	1.22	1.22	1.17	1.14
	½ hour after injection	2.80	3.70	2.70	3.12
B	Before pancreatic-jugular transfusion	1.23	1.27	1.38	1.28
	Immediately after transfusion	1.11	1.32	1.32	1.22
C	Before pancreatic-jugular transfusion	1.10	1.20	1.10	1.08
	Immediately after transfusion	0.70	1.17	0.74	0.85
	1 hour after transfusion	0.66	0.79	0.59	0.69
	2 hours after transfusion	0.70	0.81	0.66	0.74
	3 hours after transfusion	0.92	0.88	1.02	0.88
	4 hours after transfusion	0.88	1.08	1.15	0.92

prove the existence of a hypersecretion of insulin in dog B, which must be consequent to a hyperglycemia limited to the thalamic, hypothalamic and medullary regions of this dog.

If we compare these figures with those previously obtained by E. Zunz and La Barre from animals with cerebral hemispheres intact, we find little difference. We should emphasize that in control experiments which consist in the injection of the same quantity of saline in place of dextrose solution, we do not observe any hypoglycemia in the third dog. It therefore seems fair to conclude that after the removal of the cerebral hemispheres, hyperglycemia of the remaining centers greatly increases the liberation of insulin into the pancreatic venous blood. It was consequently necessary to seek the glyco-regulatory centers at lower levels.

B. Response to hyperglycemia by animals after the removal of the cerebral

hemispheres and thalamic region. In a second series of experiments, we studied the effects of hyperglycemia on animals in which the cerebral hemispheres and the thalamus were excluded by a retrothalamic section. Table 2 deals with the data obtained in four experiments of this kind. It is evident that except for the dog which receives the dextrose injection, the blood-sugar levels of the femoral bloods of B and C do not vary appreciably before and after the pancreatic-jugular anastomosis. We can therefore conclude that when the thalamic region is excluded, hyperglycemia of the remaining nervous centers does not exert a marked influence on insulin-liberation. A comparison of tables 1 and 2 seems to prove the

TABLE 2

The response to hyperglycemia after retrothalamic section in dog B

DOG	FEMORAL BLOOD REMOVED	DEXTROSE IN GRAMS PER LITER OF BLOOD IN EXPERIMENT			
		I	II	III	IV
A	Before dextrose injection	1.36	1.01	1.24	1.10
	½ hour after injection	3.40	3.20	2.28	3.52
B	Before pancreatic-jugular transfusion	1.40	1.28	1.30	1.22
	Immediately after transfusion	1.42	1.38	1.22	1.27
C	Before pancreatic-jugular transfusion	1.15	1.04	1.11	1.02
	Immediately after transfusion	1.10	0.99	1.06	1.08
	1 hour after transfusion	1.12	1.32	1.10	1.04
	2 hours after transfusion	1.15	1.32	1.13	1.10
	3 hours after transfusion	1.11	1.25	1.08	1.14
	4 hours after transfusion	1.12	1.29	1.10	1.12

existence of a thalamic center which controls the secretion of insulin during hyperglycemia restricted to the head. Actually, for technical reasons, it is not possible to give a more exact localization of these glycoregulatory centers. Since in our experiments the hypothalamus may or may not have been injured, we cannot be sure that this very important region for the regulation of circulating dextrose remained functional.

SUMMARY

The hyperinsulinema resulting from the transfusion of hyperglycemic blood through the isolated head of the dog is not affected by the removal of the cerebral hemispheres but is abolished by the separation of the thalamic region from the remainder of the central nervous system.

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THE RÔLE OF THE CENTRAL NERVOUS SYSTEM IN THE CONTROL OF PANCREATIC SECRETION

II. The External Secretion of the Pancreas during Hyperglycemia

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Babkin and Savitsch (1) observed that massive injections of dextrose which do not of themselves provoke a pancreatic secretion, increase this secretion markedly if one causes hyperglycemia in an animal the pancreatic secretion of which is kept constant by a continuous injection of secretin. Later La Barre and Destrée (2) showed that this posthyperglycemic rise in the secretory rate was caused by an excitation of the central nervous system transmitted to the pancreas by the vagi. In the work to be reported in this paper, we have tried to state more precisely the situation of the glycosensitive centers which bring about the increase in external pancreatic secretion during hyperglycemia just as we did in the case of the internal secretion. In this study it was again necessary to use a method permitting the development of an hyperglycemia restricted to the head of an animal the trunk of which was completely separated from the head except for the vagus nerves; the pancreas of this animal secreted continuously because of a coincident intravenous infusion of secretin.

Experimental results.[1] A. *External pancreatic secretion during hyperglycemia of the heads of animals without cerebral hemispheres.* The first series of experiments was devised to determine whether or not the extirpation of the cerebral hemispheres abolished the vagal stimuli to the pancreas which responds by increasing its secretion rate when the normal head is rendered hyperglycemic. After the separation of carotids, jugulars and vagi of the two dogs and the insertion of a cannula into the principal pancreatic duct (the accessory duct was ligated) the cerebral hemispheres of dog B's head were extirpated by the method described in the preceding paper. The double cross-circulation was then established between the isolated head of B and the trunk of A. A continuous and regular injection of diluted secretin was administered by way of the femoral vein of dog B

[1] One will find the details of the technique used in the present work in the paper preceding this and in the Archives internationales de Physiologie (Liége) 1929 (in press).

TABLE 1

The secretion of pancreatic juice during hyperglycemia restricted to the head after the removal of the cerebral hemispheres

EXPERIMENT	ARTERIAL BLOOD REMOVED AND PANCREATIC JUICE COLLECTED	DEXTROSE IN BLOOD (DOG A AND HEAD OF B)	PANCREATIC JUICE	
			Quantity	Lipase (in enzymatic units evaluated during periods of 10 or 15 minutes)
		gm. per liter	*cc.*	
I	Before dextrose injection			
	1st period 15 minutes		2	
	2nd period 15 minutes	1.18	2.2	3.2
	After dextrose injection			
	1st period 15 minutes		2.5	
	2nd period 15 minutes	3.18	10.05	12.5
	3rd period 15 minutes		9.20	
	4th period 15 minutes		3	
	5th period 15 minutes		2.5	6.8
II	Before dextrose injection			
	1st period 10 minutes	1.24	1.8	
	2nd period 10 minutes		1.75	5.04
	After dextrose injection			
	1st period 10 minutes		1.8	
	2nd period 10 minutes	2.36	3.9	9.94
	3rd period 10 minutes		4.8	12.00
	4th period 10 minutes		3	
	5th period 10 minutes	1.53	1.5	3.78
	6th period 10 minutes		1.5	
III	Before dextrose injection			
	1st period 10 minutes		1.2	
	2nd period 10 minutes	1.18	1.1	
	After dextrose injection			
	1st period 10 minutes		1.4	
	2nd period 10 minutes	3.08	2.4	
	3rd period 10 minutes		2.5	
	4th period 10 minutes		1.6	
	5th period 10 minutes	1.10	1.5	
	6th period 10 minutes		1.3	
IV	Before dextrose injection			
	1st period 15 minutes		3.0	
	2nd period 15 minutes	1.10	2.9	6.93
	After dextrose injection			
	1st period 15 minutes		4.5	
	2nd period 15 minutes	3.40	5.7	
	3rd period 15 minutes		3.3	
	4th period 15 minutes	2.05	3	
	5th period 15 minutes	1.58	3.2	

TABLE 1—*Concluded*

EXPERIMENT	ARTERIAL BLOOD REMOVED AND PANCREATIC JUICE COLLECTED	DEXTROSE IN BLOOD (DOG A AND HEAD OF B)	PANCREATIC JUICE	
			Quantity	Lipase (in enzymatic units evaluated during periods of 10 or 15 minutes)
		gm. per liter	*cc.*	
V	Before dextrose injection			
	1st period 15 minutes		1.6	
	2nd period 15 minutes	1.23	1.5	
	After dextrose injection			
	1st period 15 minutes		1.5	
	2nd period 15 minutes	3.80	2.1	
	3rd period 15 minutes		2.2	
	4th period 15 minutes	2.20	2.3	
	5th period 15 minutes		1.8	
	6th period 15 minutes		1.5	

and caused a constant flow of pancreatic juice during 2 to 3 hours. This constant flow was obtained ordinarily 20 to 30 minutes after the beginning of the secretin injection. Each 10 or 15 minutes during the experiment samples of the juice secreted were collected. Later, dog A, donor of the blood circulating in B's head, received by vein 10 to 12 grams of dextrose in 20 per cent solution. The dextrose-concentrations in dog A's blood were determined before and at intervals after the glucose injection. In some experiments we also estimated the lipase in the juice secreted.

Table 1 gives the results obtained during such experiments.

If we compare the results I previously obtained with P. Destrée on animals with the cerebrum intact, we see that hyperglycemia of the remaining nervous centers still produces a marked increase in juice and lipase secreted during periods of 10 or 15 minutes. The data of table 2 and data in the paper of La Barre and Destrée illustrate how little variation occurs either without the necessary nervous centers or after double vagotomy.

We must however point out that in some cases the posthyperglycemic pancreatic secretion does not increase to as great an extent as in the animals with the nervous system intact (expts. II, III, V, table 1). This decrease of the sensitivity to dextrose in these cases probably can be attributed in a large measure to a too vigorous tamponing of the cranial cavity to stop hemorrhage. Very often a rather severe pressure is necessary and causes some circulatory and trophic disturbances in the nervous tissue situated near the section line. Nevertheless we think that the degree of the posthyperglycemic increase of the pancreatic secretion is sufficient to show that the glycosensitive centers are not situated in this upper part of the encephalon. Moreover in control experiments we have also demonstraged that vagotomy performed at the moment of the marked increase

TABLE 2

The secretion of pancreatic juice during hyperglycemia restricted to the head after retrothalamic section

EXPERIMENT	ARTERIAL BLOOD REMOVED AND PANCREATIC JUICE COLLECTED	DEXTROSE IN BLOOD (DOG A AND HEAD OF B)	PANCREATIC JUICE
		grams per liter	*cc.*
I	Before dextrose injection		
	1st period 10 minutes		1.8
	2nd period 10 minutes	1.12	1.7
	After dextrose injection		
	1st period 10 minutes		1.7
	2nd period 10 minutes	3.14	1.9
	3rd period 10 minutes		1.7
	4th period 10 minutes		1.8
	5th period 10 minutes	1.94	1.8
	6th period 10 minutes		1.7
II	Before dextrose injection		
	1st period 10 minutes		2
	2nd period 10 minutes	1.28	2
	After dextrose injection		
	1st period 10 minutes		2.2
	2nd period 10 minutes	3.28	1.95
	3rd period 10 minutes		2
	4th period 10 minutes		1.95
	5th period 10 minutes	1.42	1.9
III	Before dextrose injection		
	1st period 10 minutes		1.4
	2nd period 10 minutes	1.10	1.2
	After dextrose injection		
	1st period 10 minutes		1.25
	2nd period 10 minutes	3.40	1.2
	3rd period 10 minutes		1.25
	4th period 10 minutes	1.85	1.1
	5th period 10 minutes		1.2
IV	Before dextrose injection		
	1st period 10 minutes		1.4
	2nd period 10 minutes	1.14	1.5
	After dextrose injection		
	1st period 10 minutes		1.35
	2nd period 10 minutes	3.52	1.4
	3rd period 10 minutes		1.4
	4th period 10 minutes		1.5
	5th period 10 minutes	1.85	1.4
V	Before dextrose injection		
	1st period 10 minutes		1
	2nd period 10 minutes	1.10	1
	After dextrose injection		
	1st period 10 minutes		0.9
	2nd period 10 minutes	3.10	0.8
	3rd period 10 minutes		0.9
	4th period 10 minutes	2.15	0.8
	5th period 10 minutes		1
	6th period 10 minutes		0.9
	7th period 10 minutes	1.40	0.9

in juice-flow is followed immediately by a decrease in the rate of the secretion; after 10 or 15 minutes the quantity of juice is similar to that observed before the dextrose injection.

B. *External pancreatic secretion during hyperglycemia produced by repeated dextrose injections into animals without cerebral hemispheres.* In some cases the hyperglycemia consequent to a first injection of dextrose into dog A does not persist after forty to fifty minutes and the secretion of juice increased under these conditions progressively returns to a normal level. During three experiments of this kind, we have given a second injection of dextrose when the effect of the first injection had almost disappeared; it thus is possible to produce by this second hyperglycemia a repeated sudden increase of the external pancreatic secretion. We can then conclude that a dextrose injection causes two successive increases in the flow of juice.

C. *External pancreatic secretion during hyperglycemia produced in animals without cerebral hemispheres and thalamus.* In the third series of experiments we studied the influence of hyperglycemia on the external pancreatic secretion in animals with cerebral hemispheres and thalamus excluded by a retrothalamic section. Table 2 contains the data obtained in five experiments performed as previously described. As these data show, it is not possible to observe any appreciable variation of the external pancreatic secretion when the nervous centers of the thalamic region have been previously excluded.

In a number of experiments we brought about a second phase of hyperglycemia one hour after the first injection of dextrose; but we have never observed any real modification in the pancreatic flow following this secondary hyperglycemia. It seems therefore that the exclusion of the thalamic region prevents the pancreatic hypersecretion during hyperglycemia. This is also true of the insulin-secretion. However, it is possible that the hypothalamic region situated near the section line is no longer functional. For this reason it is not possible to say positively whether the glycosensitive centers are placed exclusively in the thalamus or in the subjacent hypothalamic region.

SUMMARY

The extirpation of the cerebral hemispheres does not modify the increase of the pancreatic-juice flow consequent to hyperglycemia. Extirpation of the thalamus abolishes the response.

It is a pleasure to express my gratitude to Dr. H. B. van Dyke for the hospitality of his laboratory and for his helpful interest in this work.

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ELECTRICAL MEASUREMENTS OF NEUROMUSCULAR STATES DURING MENTAL ACTIVITIES

II. Imagination and Recollection of Various Muscular Acts

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According to the preceding investigation (Jacobson, 1930), when the subject imagines that he is steadily bending one of his arms, electrical phenomena simultaneously occur in the biceps region of that arm.[1] The aim of the present article is to extend these observations to other acts which are imagined or recalled in memory, and which, if actually performed, would involve the contraction of some skeletal muscle-group.

Practically all of the tests to be herein recounted were interspersed among those described in the foregoing article. It is convenient to describe them in groups, according to the character of the act of imagination or recollection.

Imagination of lifting a weight. The methods (including the location of electrodes) in the first series of tests to be here described are like those in the foregoing investigation except that the instruction now is, "When the first signal comes, imagine lifting a ten pound weight with your right forearm. Upon hearing the second signal, relax any muscular tensions present." Records made during relaxation compared with records made during imagination provide one type of control test. Other control tests are: control 7, in which the instruction is "not to bother to imagine" and control 2, in which the instruction is to "imagine lifting the weight with the other arm" (the arm to which the electrodes are not attached). The other types of control tests carried out in connection with imagining bending the right arm relate almost equally to imagining lifting a weight and therefore are not repeated.

Results. I. *The arm during imagination of lifting a ten pound weight: While the subject (previously trained to relax) imagines that he is lifting a ten pound weight with one arm, to which electrodes are attached, electrical fluctuations occur in the biceps region of that arm, which are absent during the foregoing and succeeding periods of relaxation.* During such imagination the

[1] Tests whether some subjects might merely visualize bending the right arm will be reported in a subsequent article.

TABLE 1

Condensed from three tables. Shows average of average results for each subject

Results are considered positive (col. 4) if the microvoltage of V°_{2m} during the test period (mental activity or control) clearly exceeds that during relaxation. Otherwise they are considered negative (col. 5) or doubtful (col. 6). The minimum and maximum values for V°_{2m} during relaxation in all the tests (number shown in col. 3) of a particular kind (col. 2) for a particular subject (col. 1) appear in columns 7 and 8, and the average value for this entire set of tests appears in column 9. These values are analogously shown for the test periods in columns 10, 11 and 12.

As will be seen, increased string vibration generally occurs during the tested mental activity (as compared with relaxation), while the control tests generally show no such increase. Compare columns 12 and 9.

Lists of the various mental activities tested will be found in the text. In tests under caption B, string is critically damped for all subjects except I. G.

In column 13 is indicated the number of times a deviation of curve, V'_m took place. If present, the minimum and maximum microvoltage of the greatest such deviation (maximum ordinate) is shown in the succeeding columns, and the general average in the last column. As in article I, the occurrence of V'_m is not found to be characteristic of imagination and recollection.

SUBJECT	CHARACTER OF TESTS	NUMBER OF TESTS	V°_{2m}									V'_m			
			Results of tests			During relaxation			During test periods			During or after test periods			
			+	−	?	Minimum	Maximum	Average	Minimum	Maximum	Average	Number of cases	Minimum	Maximum	Average
A. Imagine lifting a ten pound weight															
H.K.	Imagining	15	15	0	0	3	23	10	14	82	45	9	17+	76	55
H.K.	Controls 7	1	0	1	0	4	9	7	4	9	7	0			
B.E.	Imagining	21	20	1	0	2	20	7	3	53	21	9	3	67	22
D.M.	Imagining	11	9	2	0	2	20	7	4	64	34	4	12	30	18
L.G.	Imagining	16	16	0	0	2	20	10	16	110	54	8	7	58	27
M.J.	Imagining	26	21	5	0	4	21	13	9	181	71	21	12	320	153
R.S.	Imagining	4	4	0	0	2	16	7	11	36	25	0			
B.R.	Imagining	4	4	0	0	2	9	7	7	20	13	0			
B.J.	Imagining	8	8	0	0	2	30	13	13	64	45	0			
B.L.	Imagining	4	4	0	0	3	7	5	9	26	17	0			
B.L.	Controls 7	1	0	1	0	3	7	5	3	7	5	0			
I.G.	Imagining	6	6	0	0	2	11	8	5	60	33	4	5	39	22
I.G.	Controls 7	2	0	2	0	7	11	9	7	11	9	0			
A.K.	Imagining	5	5	0	0	4	9	7	9	62	17	5	7	14	11
A.K.	Controls 7	1	0	1	0	4	9	7	4	9	7	0			
B. Imagination of various other acts															
I.G.	Imagining	28	26	2	0	2	9	6	2	58	10	24	−7	−41	−24
I.G.	Controls 7	4	0	4	0	2	9	6	2	9	6	0			
H.K.	Imagining	42	41	1	0	2	15	7	11	110	31	0			
H.K.	Controls 7	7	0	7	0	2	15	7	2	15	7	0			
B.L.	Imagining	49	49	0	0	2	9	8	9	92	28	0			
B.L.	Controls 7	13	0	13	0	2	9	8	2	9	8	0			
B.E.	Imagining	44	43	1	0	2	7	4	9	110	24	18	−4	−35	−13
B.E.	Controls 7	17	0	17	0	2	7	4	2	7	4	0			

TABLE 1—*Concluded*

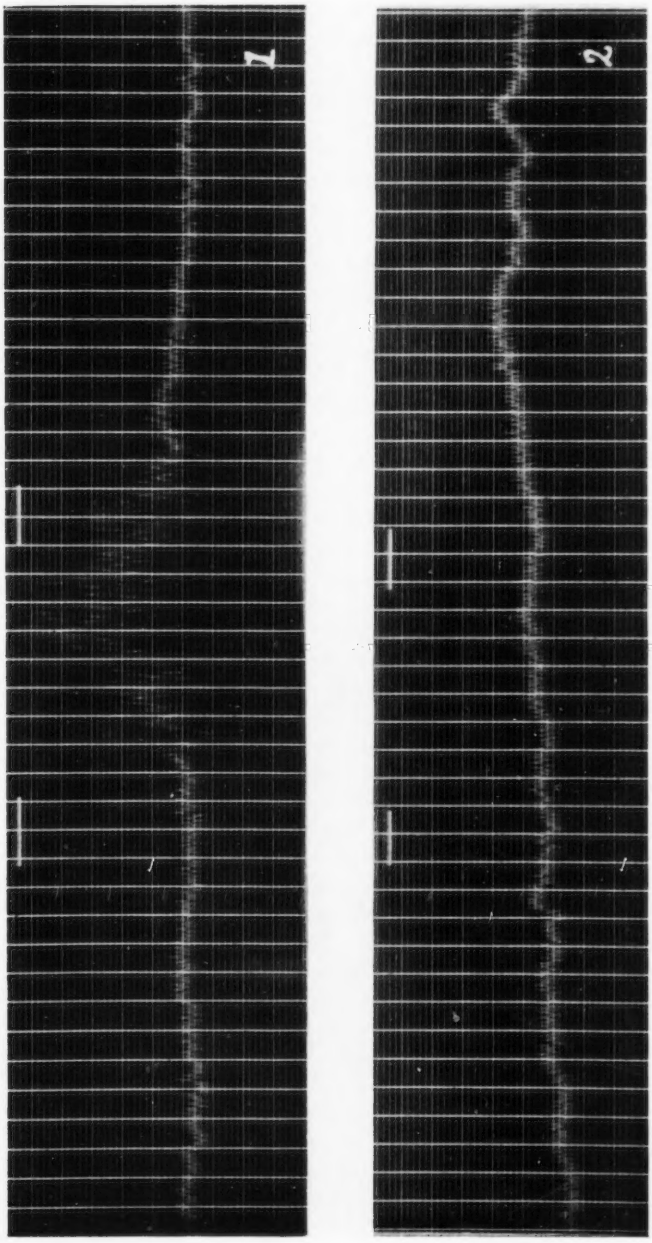
SUBJECT	CHARACTER OF TESTS	NUMBER OF TESTS	V''_{2m}									V'_m			
			Results of tests			During relaxation			During test periods			During or after test periods			
			+	−	?	Minimum	Maximum	Average	Minimum	Maximum	Average	Number of cases	Minimum	Maximum	Average
C. Recollection of various acts															
L.G.	Recollecting	41	10	28	3	4	16	10	4	30	16	1	13	13	13
M.J.	Recollecting	10	9	0	1	4	18	13	23	87	53	8	25	62	43
D.M.	Recollecting	6	3	2	1	4	27	8	4	27	13	1	23	23	23
B.R.	Recollecting	13	2	6	5	2	11	6	2	16	8	1	10	10	10
B.E.	Recollecting	25	14	10	1	2	5	4	2	76	17	4	5	21	14
B.J.	Recollecting	6	6	0	0	2	7	4	9	36	23	1	7	7	7
I.G.	Recollecting	2	2	0	0	7	12	10	14	38	25	0			
H.K.	Recollecting	21	17	3	1	4	9	7	9	87	31	0			
H.K.	Controls 7	3	0	3	0	4	9	7	4	9	7	0			
B.L.	Recollecting	20	9	6	5	1	7	3	1	29	15	3	12	12	12
B.L.	Controls 7	2	0	2	0	2	7	5	2	7	5	0			

galvanometer string behaves as was previously described for imagination of bending the arm which bears the electrodes (figs. 1 and 2). During the control tests, however, the record is like that for periods of relaxation.

Data following the instruction, "Imagine lifting a ten pound weight" are summarized under caption A in table 1. Eight of the subjects had received training to relax according to methods previously described. To rule out the possibility that such training in observing sensations or in

Fig. 1. *Photographic record of string deflections during imagination.* The subject imagines that he is lifting a ten pound weight with his right arm. Glass cups containing plain platinum electrodes in 0.9 per cent NaCl solution are attached over the right biceps region and over the coronoid fossa. Upper line: first break indicates the signal to imagine; second break indicates the signal to "relax muscularly." Before the first signal is a period of relaxation, in which the excursions of the string are relatively small, as shown by the relatively short approximately vertical lines. Vertical time lines, $\frac{1}{5}$ second. Six-tenths of a second after the beginning of the first signal, the record shows long linear deflections, which cease soon after the beginning of the second signal. Upward deflections indicate negative potential in the electrode on the biceps as compared with the other electrode. Distance between parallel horizontal lines is 1 mm. $= 2.3 \times 10^{-6}$ volts. No thermostatic regulation. Magnification about 600. String not critically damped. Subject, I. G., medical student, not trained to relax.

Fig. 2. *Photographic record of control test.* Conditions the same as for figure 1, but the subject has been instructed "not to bother to imagine" when he hears the first signal. No electrical change now takes place following the first signal.



Figs. 1 and 2

relaxing had in some manner influenced their records, the results from three untrained subjects (R. S., A. K., and I. G.) are likewise included in the table.

The results for all subjects are positive in approximately 93 per cent of the tests (120) during such imagination. A result is classed as positive if the magnitude of the string vibrations during imagination clearly and considerably exceeds that during relaxation. The control tests generally proved negative, including five records with several subjects instructed as stated above for control series 7. Since the signal was present in these control tests which proved negative it is confirmed that the sound does not of itself act as a sufficient stimulus to produce increase of string vibration.

Following the signal to imagine lifting the weight, the deflections V°_{2m} greatly increase, reaching a value for the various subjects ranging approximately from about 186 to 550 per cent of that during complete relaxation. For most of the subjects (9 out of 11), the value during imagination is 300 per cent or more of that during relaxation. For all the subjects averaged together, the value during imagination is about 450 per cent of that during relaxation (41 microvolts/9 microvolts). This may be compared with the value found in the preceding article for imagining bending the right arm as compared with relaxation (37 microvolts / 10 microvolts). The former ratio exceeds the latter ratio by about 23 per cent. Possibly this indicates that action-potentials during imagination of lifting a ten-pound weight tend to be higher in microvoltage, as measured by present methods, than action-potentials during imagination merely of bending the arm.

The long linear deflections, which appear after the signal to imagine, promptly subside following the signal to relax muscularly. This holds, as in the preceding experiments, for the trained subjects in practically all instances and for all the untrained subjects with exceptional instances previously discussed. In both sets of tests all subjects report that imagination ceased after the signal to relax and all agree that the experience of imagination of lifting a ten pound weight has as an essential component a sensation as of extremely slight muscular tenseness in the biceps region.

Prolonged rises or falls of curve V'_m occur in half the total number of tests with all subjects, 60 out of 120. About the same proportion was found in the preceding article in connection with imagining bending the right arm.

II. *Reaction and relaxation times.* In order to condense this article, temporal tables have been omitted. Reaction times were legible in the records in a representative number of cases, 55 in all; in others they were not ascertained, owing to obscuring or failure of signals or to the absence of a clearly marked point where the observed increase of linear deflections could be definitely stated to begin. In the majority of the legible instances (36 out of 55), the period from the sound of the buzzer to the initiation of

the long vibrations V°_{2m} of the string varies from 0.2 to 0.8 second inclusive. This range likewise applies to the majority group of subjects (7 out of 10) in most of their tests (34 out of 48). It applies likewise to two out of three of the other subjects in half of the instances. Accordingly the electrical changes V°_{2m} occurring during imagination, have a different time of occurrence from the psychogalvanic reflex, which, as all investigators agree, follows the stimulus after an interval of at least one to four seconds.

It seems unnecessary to present complete temporal data for the occurrence of V'_{m}, since the present data are in entire harmony with the results in the preceding article.

Following the onset of the signal to relax, there generally occurs, with subjects who have been trained to relax, a distinct diminution of the large deflections V°_{2m} within 0.1 to 1.1 second, averaging 0.4 second. The complete disappearance of such deflections under the same conditions takes about 2 seconds on the average; but in 3 cases out of 19 it exceeded 6 seconds for trained subjects, and in 6 cases out of 13 it exceeded 6 seconds for untrained subjects.

IMAGINATION OF VARIOUS ACTS COMMONLY PERFORMED WITH THE RIGHT ARM. The foregoing results, together with those of the preceding article, afford evidence that imagination of a muscular act includes the presence of action-potentials in some of the muscles whose contraction would be required in the actual performance. Instructions thus far have been clear and definite as to the limb and muscle-group which are to be imagined as bending or lifting a weight. But it is necessary to test for the presence of action-potentials when instructions are given which can be carried out by the subject in terms of responses of one or another muscular group not always determinable in advance. Ideally, for these tests we should need a separate string and a set of electrodes for each important neuromuscular group and a galvanometer circuit considerably freer from inductance effects than is the present circuit; for there is always the possibility that results may be called negative through masking of very slight action-potentials by extraneous electrical disturbances.

In preliminary tests, the following instructions were selected by the experimentor (without consulting the subject beforehand): "When the signal comes, imagine sweeping a room with a broom. Upon the second signal relax any muscular tensions present." Other examples of instructions are: "Imagine writing your name"; "Imagine yourself rowing a boat"; "Imagine yourself boxing"; "Imagine scratching your chin"; "Imagine plucking a flower from a bush"; "Imagine combing your hair"; "Imagine playing the piano."

In 28 such tests, 14 disclose action-potentials from the biceps region, having an average microvoltage of 20 for all subjects. This is twice the average microvoltage during relaxation; but it is considerably less than the

average microvoltage during imagination of bending the right arm (20 microvolts / 37 microvolts).

Long linear deflections $V°_{2m}$ are found to be fewer and in briefer series in the positive results of the records of the present section than in the records for imagination of bending the right arm or lifting a ten pound weight.

In this series, V'_m occurs in 11 out of 28 cases and has a total average microvoltage of 29. This value is close to that secured in records of imagining bending the right arm.

The subjects sometimes stated that following the instruction to imagine, they carried out the imagined act with some muscle-group other than the flexors of the forearm. In other instances they stated that they visualized themselves performing the act, but failed to have arm-muscle sensations. If these statements are correct, they evidently account for the absence of action-potentials from the biceps region in some of the above described tests.

If the instructions selected by the experimentor fail in some instances to induce the subject to imagine various muscular acts as carried out with his right biceps, but lead him instead to visualize or to carry out the imagination with other muscle-groups, another approach is suggested: The subject is asked what various acts he would naturally perform with the right arm and would naturally imagine as so performed. In making such selection, he will be aided if he has had experience at observation of the muscle-sense (Jacobson, 1929). Four subjects were used in the following tests who had such experience. In addition, all of them had been trained to relax, except I. G.

The instructions selected by them were as follows: "Imagine lifting a cigarette to your mouth"; "Imagine pulling a microscope toward yourself"; "Imagine pulling up your socks"; "Imagine grinding coffee"; "Imagine yourself chinning on a horizontal bar"; "Imagine hugging"; "Imagine shifting the gear of your automobile to first speed"; "Imagine pulling a door open"; "Imagine lifting a glass of milk to your mouth"; "Imagine climbing a rope"; "Imagine throwing a ball"; "Imagine pulling up weeds"; "Imagine pumping a bicycle tire."

Results for these four subjects are under caption B in table 1. During imagination, results are positive for action-potentials from the right biceps-brachial region in 97.5 per cent of the tests (159 out of 163). All of the control tests proved negative. These included 41 control tests in which the subject was instructed either not to bother to imagine when the signal came or to imagine the same act performed only with the other arm (the arm to which the electrodes were not attached).

The microvoltage for such imagination as averaged for all subjects is

about 26. This is much less than the average value found for imagining lifting a ten pound weight (41 microvolts).

Beautiful records were secured when the subject was requested to imagine some act performed rhythmically such as "climbing a rope" or "pumping a tire" or "turning an ice-cream freezer." Following the signal to imagine, the string shows a brief period of long vibrations, then an interval of rest for a fraction of a second or more, then similar periods of vibrations and rest, occurring in rhythmical succession until the signal to relax is given. On the other hand, instructions to imagine some act that involves only one relatively brief muscular action, such as "throwing a ball," typically are followed by only one brief series of long vibrations. But if the instruction is varied, "When the signal comes, imagine throwing a ball. After an interval, repeat this experience," then the record shows two periods of long vibrations, separated by an interval of rest (figs. 3, 4). Corresponding results follow the instruction to imagine the act three times.

Reaction times are entirely consistent with what has previously been said for reaction times for imagination of bending the right arm. Relaxation of imagination, as marked by complete disappearance of long vibrations following the second signal, is complete on the average for the trained subjects in less than 0.3 second, as compared with 2.0 seconds for the untrained subject.

RECOLLECTION OF VARIOUS MUSCULAR ACTS COMMONLY PERFORMED WITH THE RIGHT ARM. The aim of the studies of the present section is to test for the presence of action-potentials during recollection. Instructions are to recall such activities as lifting a glass or a cup of tea at dinner, reading a certain paper, removing a shirt, putting on a coat or rubbers, raising the right arm, picking flowers, pulling up dandelions, pulling up an augur, playing the piano, playing the violin, boxing, punching a bag, brushing teeth, washing dishes, sweeping, running a carpet-sweeper, scrubbing, rowing, hitting a ball at tennis, fishing, landing a fish, slinging a sledge. The subject stated before or after each test that he had previously had the experience denoted by the instruction.

If instructions are so chosen by the experimentor as to indicate an activity commonly performed by the right arm, we may reasonably expect that if recollection involves neuromuscular activity, some noteworthy proportion of responses will take place in the right biceps-brachial region and therefore be recorded. If instructions are chosen by the subject to indicate an activity which he observes that he recalls with muscle-sensations from the right arm, we should expect a higher proportion of positive results, in accordance with what was found above (under caption B, table 1) for imagination.

Results secured by the former procedure are summarized under caption C in table 1 for 8 subjects trained to relax and 1 subject not so trained.

For 7 of these 9 subjects (the two exceptions being B. R. and L. G.) the results predominantly show action-potentials from the biceps-brachial region, that is, in 60 out of 90 tests. For 6 subjects the microvoltage averages a little under 21, which is about 350 per cent of the average microvoltage (6) during the relaxation periods of these same subjects. This microvoltage during recollection is about equal to that during the various acts of imagination described in the preceding section. But as a rule, the action-potentials do not persist so long as during imagination of bending the right arm or lifting a ten pound weight. During recollection they may appear for only a small fraction of a second, disappearing and then reappearing perhaps once or more times in a brief series A very precise correspondence between the photographic record and the subjective occurrence is clearly shown in certain records For example, one subject volunteered that he clearly recalled grinding coffee at his home during his childhood A record of this recollection is shown in figure 5. The actual performance of grinding coffee obviously involves the rhythmical contraction and relaxation of the biceps-brachial muscle-group. On the photographic record the rhythmical recurrence of action-potentials from this muscle-group is shown by the regular appearance and disappearance of the lines representing increased string vibrations. Somewhat similar records indicating rhythmical activity were secured with another subject following the instruction to recall brushing his teeth.

With two of nine subjects, lengthened string vibrations occur during

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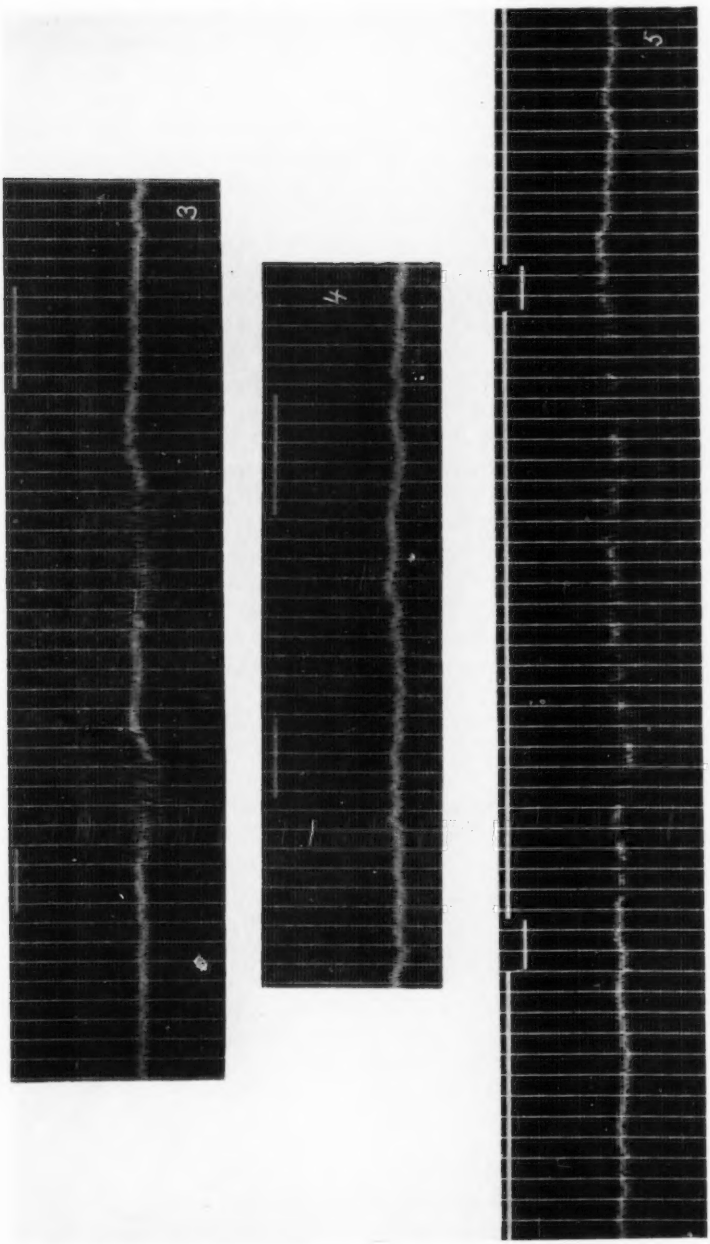
Fig. 3. *Photographic record of string deflections during imagination.* First break in signal line at top of photograph indicates the signal to "imagine throwing a ball twice;" second break indicates signal to "relax muscularly." Before the first signal is the period of relaxation, in which the string is seen to be relatively quiet. Five tenths of a second after the beginning of the first signal, the record shows long linear deflections, lasting about 1.4 second, then rest for about one second, then a second series of long linear deflections lasting about 1.4 second with a few stragglers in the following 0.4 second. The subject has already relaxed before the signal to relax occurs. String critically damped; 1 mm. = 2.2×10^{-6} volts. Downward deflection indicates negative potential of the electrode on the biceps compared with the other electrode. Subject B. L., trained to relax.

Fig. 4. *Photographic record of control test.* Conditions the same as for figure 3, but the subject has been instructed "not to bother to imagine" when she hears the first signal. No electrical change now takes place following the first signal. Subject B. L. Control records of similar appearance, showing negative results, are obtained with subject B. E. when the instruction is given to imagine performing the act with the other arm,—that is, the arm to which the electrodes are not attached.

Fig. 5. *Photographic record of string deflections during recollection.* Upon hearing the first signal, the subject recalls grinding coffee as a child. The periodic recurrence of long linear deflections evidently corresponds with the rhythmical activity of the biceps during coffee grinding and furnishes a beautiful example of the possibility of securing an objective record of a mental act; 1 mm. = 2.3×10^{-6} volts. No thermostatic regulation. Subject B. E., trained to relax.



Figs. 3 to 5

recollection in only 12 of 54 tests. One of these two reported that her experiences of recollection were nearly all visual and not motor in type; that is, not like her experience in imagining lifting the weight, which showed marked action-potentials. The other subject frequently reported that he saw himself perform the act and that in some tests the experience was mainly visual. It is suggested that the most suitable method to test for peripheral responses during recollection is to inquire of the subject in advance as to what instructions shall be given, as was done for the revised tests on imagination presented under caption B in table 1. But since recollection is evidently similar to imagination of the same muscular acts, it seems safe to assume that the revised procedure performed for imagination would analogously secure a high proportion of positive results if applied to recollection.

Deviations in curve V'_m occur infrequently in the present records of recollection,—but 19 out of 144 cases and require no special consideration. A relative diminution of electrical response of all types is noted in the present tests on recollection as compared with imagination of bending the arm or lifting a weight. This is in harmony with reports from the subjects that recollection as an experience is more fleeting and vague than imagining steadily bending the right arm or lifting a ten pound weight.

With the present circuit, inductance effects prove unavoidable even under the best conditions. Generally, their wave forms are distinguishable from those due to physiological phenomena. But there is danger that very slight potential changes, such as those due to recollection, may in some instances be obscured. Accordingly, negative results do not make certain that action-potentials are altogether absent in the region tested but only that they are not marked enough to be detected under the present limitations.

Discussion. In the present studies and those to follow, the attempt is made to secure measurements of neuromuscular states during the chief types of imagination, recollection, and other mental activities. It seems possible, with care, to select representative samples and to classify them according to the muscle-group to be electrically tested during their presence. Obviously there are many conceivable manners of classifying mental activities which would be of no interest from the standpoint of the present physiological studies. But in the foregoing tests, including those of the preceding article, an instance of imagination was first selected for study illustrative of a type of mental activity familiar and common in its occurrence in everyday life. In this type of mental activity, the individual imagines that he is performing some act through contraction of his voluntary musculature and is deliberately conscious of the contraction. Several other sample tests have been made with electrodes suitably attached to muscle-regions of the lower limbs. The instructions have been

to "imagine bending the leg" or "imagine walking." During imagination under these conditions, results have been positive for action-potentials in certain muscles whose contraction would take place in actually performing the act. It appears reasonable to generalize that the conclusions which hold for imagining bending the right arm apply analogously to the imagined contraction of all other striated muscle-groups, including the acts of flexion or extension at other joints.

A further step has been taken in the present article, where samples have been studied of imagination of various muscular acts. In these the instruction has been worded so as to lead the subject to be conscious not of his musculature but rather of the operation to be performed. Another step has included the extension of these studies to recollection under similar conditions. Accordingly, the finding of electrical evidence of neuromuscular activity during the above-mentioned three types of mental activity already makes probable the widespread character of the neuromuscular element in everyday mental life. During a period of study of relaxation covering twenty years, individuals who have been trained to observe their muscle-sense have agreed in independently reporting sensations as from slight or pronounced muscular contraction marking the occurrence of mental activity. The writer has repeatedly made this observation during the above-mentioned period. Accordingly, it now appears entirely possible that the muscle-sense, if carefully and critically employed, may put us on the track of observations that may be objectively verified for the whole range of our mental experience. The aim of the present investigations is to achieve as complete as possible an objective line of evidence.

Differences between the psychogalvanic reflex (P. R.) and the markedly elongated V°_{2m} here found characteristic of imagination and recollection are now restated: 1. *Voltage:* P. R. has many times the voltage of V°_{2m} and is commonly recorded with the D'Arsonval galvanometer, which is not sensitive enough to record V°_{2m}; 2. *Reaction-time:* An interval of 1 to 4 seconds, or more, commonly intervenes between the stimulus and the occurrence of P. R. But only a fraction of a second commonly intervenes between the signal to imagine and elongation of V°_{2m}. Evidence is presented that the latter phenomena are simultaneous with imagination. 3. *Frequency per second:* P. R. commonly lasts considerably more than 1 second, while V°_{2m} occurs frequently per second. 4. *Wave form:* P. R. is a rise or fall of curve, while V°_{2m} at usual film rates is a series of connecting almost vertical lines. Photographic records of V°_{2m} during imagination resemble those taken with similar attachments when the subject bends his arm a centimeter or more, excepting the above mentioned difference in voltage. 5. *Direction of potential:* P. R. often is chiefly unidirectional, with respect to the line of zero potential. But in all instances either electrode used in recording V°_{2m} is alternately positive and negative fre-

quently per second. 6. *Tissue of origin:* P. R. arises from changes within the skin,—sweat gland secretion, vascular changes, or altered permeability of cells, while $V°_{2m}$ can be recorded directly from muscular tissue, if the skin has been excluded. 7. *Stimulus:* For P. R., an exciting sensory stimulus or some word is selected to arouse the emotions. The reaction is involuntary. For $V°_{2m}$, a particular instruction is given to imagine or recollect, and the subject carries it out and ceases at will.

CONCLUSIONS

1. With imagination and recollection of voluntary acts there characteristically occur measurable electrical changes in neuromuscular regions which would participate or did participate in the actual performance of the same act.

2. The action-potentials that are here found characteristic of imagination and recollection of muscular acts are readily distinguished from the psychogalvanic reflex because of marked differences in voltage, reaction-time, frequency per second, wave-form, direction of potential, tissue of origin, and stimulus.

3. Relaxation of the neuromuscular processes characteristic of the occurrence of voluntary conscious activities such as imagination of lifting a ten pound weight is accomplished within 0.3 to 0.4 second on the average in the present experiments. It is confirmed that diminution of conscious activities can be brought about by muscular relaxation (Jacobson, 1925, 1929, 1930).

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THE RÔLE OF GLYCOGEN IN THE CONTRACTION OF THE PERFUSED HEART OF THE RABBIT

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The experiments here reported were undertaken for the purpose of determining the factors responsible for the ultimate failure of the hypertrophied heart in conditions of experimental myocardial failure. As a starting point for this investigation it was considered advisable to study the influence of such factors as the glycogen content, the oxygen consumption and the total work of the heart under normal and abnormal conditions.

Since, in the Hill-Meyerhof theory of muscular contraction, glycogen plays a predominant part, it was considered advisable at the outset of the research to determine the survival period of the heart relatively devoid of glycogen when perfused with glucose-free Ringer-Locke's solution. The results obtained have been sufficiently definite to justify the present report.

EXPERIMENTAL METHODS. The hearts of rabbits were perfused after the manner of Locke and Rosenheim (1907–1908). The animal was etherized and bled to death by transection of the abdominal aorta and vena cava. The heart was gently excised without injury to the auricles, was rinsed with glucose-free Ringer-Locke's solution until free of blood and then was perfused with the well oxygenated liquid at a hydrostatic head of 70 cm. and at a temperature of 37°C. The technic for eliminating glycogen from the hearts of rabbits was as follows: The animal was fasted for three days and then 0.4 mgm. of strychnine sulphate for each kilogram of body weight was injected intravenously to render the animal hyperexcitable. Sometimes small doses of strychnine were repeatedly injected subcutaneously and definite manifestations of the desired severity and duration could be readily induced. During prolonged spasm of the respiratory muscles artificial respiration was administered. We found it possible to maintain rabbits in this state for two to three hours. At the end of this time the animal was etherized and bled to death. The heart was then excised, rinsed free of blood, mopped gently in the folds of a towel and weighed. For the estimation of glycogen it was dropped into an equal volume of boiling 60 per cent potassium hydroxide.

In view of the fact that traumatized muscle explosively decomposes its glycogen into lactic acid, it was considered essential not to cut the muscle of the heart into small pieces. Glycogen was estimated by the well known Pflüger method, with certain minor modifications. To control the accuracy of the analyses, on several occasions an equivalent quantity of a standard solution of glycogen was similarly treated and the quantity recovered was always more than 90 per cent of the theoretic value. After hydrolysis of the glycogen the sugar was estimated by the Shaffer-Hartmann method and the copper reduction values were transcribed to glucose by means of Scott and Duggan's tables. The figure was then multiplied by the factor 0.927 to change the value to glycogen. Occasionally duplicate determinations were made with good agreement by means of two aliquot samples of the potassium hydroxide digest.

RESULTS. The content of glycogen in the hearts of seven rabbits fasted at room temperature for three days varied between 0.33 and 0.50 per cent, with an average value of 0.40 per cent. The content of glycogen in the hearts of seven rabbits fasted for four days varied between 0.11 and 0.43 per cent, with an average of 0.25 per cent. Table 1 shows the content of glycogen in the hearts of fasted rabbits subjected to increasingly severe convulsions from strychnine. It will be seen that after two hours of intermittent tetanus from strychnine the content of glycogen in the heart is always less than 0.1 per cent. Accordingly, we perfused two sets of hearts with glucose-free Ringer-Locke's solution: 1, the hearts of well fed rabbits and of rabbits fasted for three days, and 2, the hearts of rabbits fasted for three days and subjected to tetanus from strychnine for at least two hours.

Under the conditions of our experiments the hearts of normal and fasted rabbits, perfused with well oxygenated Ringer-Locke's solution containing glucose, function in good condition for about eight hours, barring the occasional experiment that was unsuccessful because of some flaw in technic. As is now well known from the memorable work of Locke and Rosenheim, the absence of glucose from such a fluid materially diminishes its value as a perfusing medium. Since it was essential for the clarity of our experiments that the perfusing fluid should not contain a substance that could serve as nutriment to the heart, we deliberately omitted the glucose from the perfusing fluid. The survival period of a rabbit heart so perfused is about four hours, and the beat is vigorous most of the time. The survival period and vigor of contraction are not significantly less whether the heart be almost free of glycogen on the one hand or rich in glycogen on the other. Tables 2 and 3 summarize the actual results obtained.

The perfusion was terminated when the heart beat was barely visible but was still clearly perceptible to touch. At this time it could be considered that the heart was functioning sufficiently to permit an analysis of its glycogen. Since the hearts were weighed at the beginning of the experi-

ments the percentage of glycogen in the heart refers to the initial weight and not to the weight of the heart before being dropped into caustic potash. Such hearts of course are always edematous. Hearts containing normal amounts of glycogen at the beginning of the perfusion showed the greatest loss in glycogen (table 2).

We found it impossible to rid the heart of every trace of glycogen. When the precaution is taken to handle the heart gently and not to cut it up into bits before adding it to alkali, it always contains glycogen.

TABLE 1

Glycogen in unperfused heart of rabbits fasted three to six days and subjected to convulsions from strychnine

RABBIT	DAYS OF FAST	PERIOD OF CONVULSIONS		GLYCOGEN IN HEART, PERCENTAGE
		hours	*minutes*	
1	3		3	0.24
2	3		10	0.18
3	6		30	0.13
4	3		40	0.16
5	3		45	0.07
6	3		45	0.19
7	6		45	0.15
8	4	1		0.05
9	3	1		0.14
10	3	1		0.14
11	4	1	10	0.15
12	4	1	15	0.08
13	3	1	30	0.25
14	3	1	40	0.10
15	3	1	40	0.08
16	3	1	40	0.07
17	3	2		0.06
18	3	2	30	0.09
19	5	2	30	0.09
20	4	3		0.06
21	3	3		0.09

Various types of treatment are given in the literature which can lower or, according to some observers, deplete completely the heart and other organs of glycogen. Among these are 1, the feeding of thyroxine; this we did not try because of the other effects that thyroxine might have on the heart; 2, convulsions from strychnine (or insulin) associated or not with cold and fasting, and 3, the combined injection of phlorizin and epinephrine. Phlorizin and epinephrine were given in accordance with the instructions of Sansum and Woodyatt. Glycogen was always present in the heart and occasionally its concentration was not even low, as shown by the results in four rabbits, as follows:

Rabbit 36, on the fifth day of the fast, was given 2 grams of phlorizin subcutaneously, on the sixth day, 2 grams of phlorizin subcutaneously, and on the seventh day, 1 gram of phlorizin subcutaneously. Soon afterward, five injections of 0.15 cc. of epinephrine 1:1000 were given every three hours intramuscularly. Every six hours Fischer's solution was injected intravenously; copious diuresis occurred. The animal was killed forty-five

TABLE 2

Hearts of rabbits perfused with glucose-free Ringer-Locke's solution

RABBIT*	PERIOD OF SURVIVAL		GLYCOGEN AT THE END OF PERFUSION, PERCENTAGE
	hours	*minutes*	
22	4	10	0.13
23	4	45	0.04
24	6	20	0.12
25	1	35	0.13
26	3	45	0.11
27	3	20	0.09
28	2	00	0.13
29	6	00	0.10
30	4	05	0.06
31	4	00	0.03

* Rabbits 22, 23 and 24 were wel l fed and, rabbits 25 to 31 were fasted three days.

TABLE 3

Hearts of rabbits perfused with glucose-free Ringer-Locke's solution. The animals were fasted three days and subjected to convulsions from strychnine

RABBIT	PERIOD OF				GLYCOGEN AT THE END OF PERFUSION, PERCENTAGE
	Convulsions		Survival		
	hours	*minutes*	*hours*	*minutes*	
32	2	10	3	10	0.07
33	2		4	10	0.05
34	3		4	15	0.03
35	2	30	4	30	0.07

minutes after the last injection of epinephrine, and the content of glycogen in the heart was 0.14 per cent, and in the liver 0.04 per cent.

Rabbit 37, on the fourth day of the fast, was given 1 gram of phlorizin subcutaneously, on the fifth day, 1 gram of phlorizin subcutaneously, and on the sixth day, 0.5 gram of phlorizin subcutaneously. Soon afterward, three injections of 0.2 cc. epinephrine 1:1000 were given every three hours intramuscularly. The animal was killed thirty minutes after the last injection of epinephrine, and the content of glycogen in the heart was 0.06 per cent and in the liver 0.05 per cent.

Rabbits 38 and 39 were given the same treatment as was rabbit 37, and the content of glycogen in the heart was 0.1 and 0.21, respectively.

The results presented in this paper therefore show rather clearly that if glycogen is present in considerable quantities in a perfused heart, much of it has disappeared at the end of perfusion, but if very little is present in such a heart, the heart beats as vigorously, and a considerably smaller amount of glycogen disappears. It would seem that if glycogen is present in a heart it is used by preference, but if it is absent some other foodstuff can serve as a source of energy.

Locke and Rosenheim found that 1.2 to 1.7 mgm. of glucose for each gram of heart muscle each hour disappeared from the perfusion fluid, and in the hands of numerous other workers this figure has never been lower than 0.5 mgm. Assuming that this glucose is combusted and does not become transformed into glycogen or something else, one gets an approximate idea of the nutritional requirements of a perfused heart under the experimental conditions employed by us.

We have not found data in the literature regarding the oxygen consumption of a heart perfused under our experimental conditions; most of the experiments recorded in the literature were done either on the heart-lung preparations, or on hearts subjected to varying degrees of tension. The production of carbon dioxide in the experiments of Locke and Rosenheim is of interest in this connection because these experiments are not too dissimilar. In two cases in which hearts of rabbits were perfused, that is, 1, without calcium, and 2, without calcium and potassium, the production of carbon dioxide was 0.56 and 0.35 cc. for each gram of heart muscle each hour respectively. If the heart was perfused in the usual manner, that is, with Locke's solution containing glucose, the production of carbon dioxide was 0.64 cc. for each gram of muscle each hour. Assuming that these data are not too divergent from what would be obtained in a heart perfused under our experimental conditions, one can readily see that the glycogen which disappears from a heart deficient in glycogen in our experiments was considerably less than would be required if glycogen were the sole foodstuff for muscular contraction. It is necessary in this connection to note the work of Hines, Katz, and Long. They found that the amount of lactic acid produced in caffein rigor of heart muscle was occasionally twice as great as the content of glycogen in the muscle. These workers raised the possibility that perhaps heart muscle differed from skeletal muscle in possessing an additional precursor for lactic acid. Our results may also be compared with those of Olmsted and Coulthard who found that the fatigue curve of a practically glycogen-free gastrocnemius muscle of the frog was not significantly different from a gastrocnemius muscle containing a normal amount of glycogen.

It is well known that the presence of glucose materially improves the

value of Ringer-Locke's solution as a perfusing fluid for the heart. Since a perfused heart that is poor in glycogen beats as efficiently as a heart containing the usual quantity of glycogen, it is clear that the glucose does not exert its beneficial influence by being transformed into glycogen.

CONCLUSIONS

1. Hearts of rabbits containing normal amounts of glycogen (0.4 per cent after three days of fasting) show a marked fall in glycogen if they are perfused with glucose-free Ringer-Locke's solution.

2. Hearts containing traces of glycogen (less than 0.1 per cent) and perfused identically have approximately the same survival period of about four hours.

3. The consumption of energy of the perfused heart is believed to be sufficiently great so as to preclude the possibility that hearts deficient in glycogen can obtain their energy from the oxidation of the remaining traces of this substance.

4. It would appear from these observations that when glycogen is present in a heart, it is used by preference, but that when it is gone, some other foodstuff can serve as a source of energy.

5. The value of glucose in the perfusing fluid for the heart is considered. On the basis of our observations, it is suggested that the beneficial effect of glucose is not due to the fact that it is converted into glycogen.

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COMPARATIVE STUDIES ON THE PERIPHERAL AND CENTRAL RETINA

I. On Interaction between Distant Areas in the Human Eye

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Adrian and Matthews (1) have recently demonstrated the interaction of retinal neurones by recording the impulses in the optic nerve of the Conger eel. The evidence for the occurrence of interaction has been summarized by them as follows: 1, "When the entire retina of the Conger eel is exposed to uniform illumination the action current discharge in the optic nerve may lose its usual irregular character and may consist of a series of regular waves . . . . caused by a rhythmical waxing and waning in the number of impulses in the nerve fibres. It follows that the ganglion cells of the retina must all be working in unison with alternating periods of rest and activity." 2, "when four points on the retina are illuminated simultaneously, the reaction time of the optic nerve discharge is shorter than when any one of the points is illuminated alone." This effect was similar to the one following an increase in the intensity of the stimulus. That it most probably is due to spatial summation of the type met with in reflex arcs was shown by the fact 3, that strychnine caused a marked increase in the range of area over which interaction occurred.

There is much evidence (see Parsons (2) pp. 103 and 130) which tends to show that also in the human eye spatial effects are easily obtained. For instance, it has been found that under certain conditions an increase of the area excited has an effect similar to that resulting from an increase in the intensity of the stimulus. An important link in the chain of evidence needed for allowing these experiments to be set in parallel with those of Adrian and Matthews (1) would seem to be within reach, if it were found that in the human eye distant areas affected one another in the way indicated by their experiment with the four test patches.

In order to apply this experiment to a visual test some method had to be used which would allow different degrees of brightness to be measured without the aid of a comparison light. This is possible with the flicker method, which also is accurate enough for the purpose in question. With this method an alteration of the physiological effect in the excited area

similar to the one following an increase in the intensity of the stimulus will raise the fusion frequency of an intermittent light. Such a relation, besides, was found by Adrian and Matthews (1) in some experiments with a flickering light to apply also to the excised eye of the Conger eel.

METHOD. A disc with two symmetrical opaque sectors of 90° each was rotated in a light beam projected on to a ground glass. This glass was covered on the back by a black paper, having a circular opening for the light. Two black metal discs having four circular holes of 1° of diameter, arranged as shown in figure 1, could be fitted to cover this opening. The distances between the holes in the two plates (*I, II*) are given in figure 1 in terms of visual angles. The fixation point, a thread cross, was either in the middle, *F*, or 10° out towards the periphery, F_1, and was viewed binocularly at a distance of 50 cm.

To the axle of the motor used for rotating the opaque sectors was joined a Weston electric tachometer, accurate to less than 1 per cent and cali-

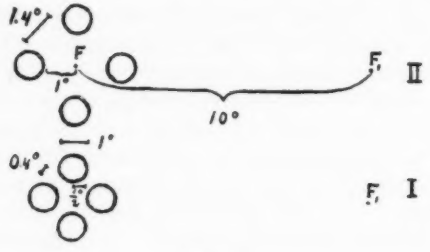

Fig. 1

brated by means of a revolution counter and an interposed speed reducing system. A Mazda lamp of the concentrated filament type was used as light source and the voltage supplied to it from the mains was kept at 110 v. The lamp was calibrated against a standard in a Lummer-Brodhun photometer and at the distance used projected 94.0 meter candles on to the ground glass. With a Tscherning neutral tint glass no. 2 the brightness of the test patches could be reduced 100 times. The background throughout the experiments was at a brightness of 0.0005 that of the stimulus (94.0 m.c.) but bright enough not to allow the light scattered by the diffusing ground glass to show around the test patches. The adaptation of the eye was at about 0.032 millilambert, as determined from the average size of the pupil of three observers and the standard values published by Reeves (3).

The experiments were always begun by taking two readings for each of the test patches viewed in turn, followed by three or four double readings for the four patches together. With arrangement I in the periphery,

simultaneous observation of all the patches showed but a small mean variation which for all observers averaged 0.08 revolution per second; for the single patches this value was larger, viz., 0.17, as is only natural in view of the different localization of the spots. Long fixation was avoided in the periphery because of its great adaptability. In two seconds the value may easily decrease as much as 1 revolution per second.

Results. In table 1 the results with five subjects are summarized. The average fusion frequencies in revolution per second for the single test patches and for all of them together as well as the corresponding mean variations (within brackets) are given on the right of each column; on the left will be found the maximum and minimum values obtained. The figures I and II indicate the grouping of the patches as shown by figure 1. The terms "singles" and "fours" will be used as convenient abbreviations in referring to the columns headed "single patches" and "all together" respectively.

With the higher intensity and arrangement I there is evidently a great deal of interaction taking place in the periphery. The fusion frequencies are regularly from 1.6 to 3.1 (average for all subjects 2.5) revolutions per second higher than those of the singles. With central fixation and the spots falling on the edge of the fovea and on the paracentral area there is much less summation under otherwise similar conditions. Observer D. G. shows no signs of interaction in the centre; she had little practice with flicker. The regular observers have an average increase of 0.24 for the fours over the singles. This value is greater than the mean variation, but, on the other hand, the maximal value for the singles may reach the average value for the fours. Evidence derived from experiments to be published shortly tends to show that this slight increase in value for the central fours, as compared with the singles, actually is caused by slight interaction between the stimuli.

With the 100 times lower intensity there still remains some interaction in the periphery, though the increment in fusion frequency, when all four patches are taken together, is much less, only about 0.6 revolution per second, as compared with 2.5 for the same subjects with the brighter stimulus. In the centre there seems to be no summation at all.

Turning to arrangement II, where the patches are further apart, we find an average increase for the fours over the singles amounting to about 1.6 revolutions per second with the higher intensity. This means that doubling the radius of the circle on the circumference of which the patches lie and more than trebling the shortest distance between them does not diminish the interaction between them by more than about 46 per cent. In two out of three cases there is a definite although slight interaction with the weaker intensity.

From these observations it will be seen that there is a very striking

TABLE 1

SUBJECT	METER CANDLES	ARRANGEMENT I				ARRANGEMENT II	
		Peripheral fixation		Central fixation		Peripheral fixation	
		Single patches	All together	Single patches	All together	Single patches	All together
R. G.	94.0	23.12	24.25	25.10	24.96	22.56	23.41
		22.56	24.25	24.79	24.87	22.05	23.27
		(0.28)	(−)	(0.17)	(0.06)	(0.25)	(0.08)
		22.48	24.25	24.53	24.82	21.71	23.27
	0.94	14.95	15.51	15.79	15.79	14.95	15.09
		14.58	15.28	15.54	15.71	14.69	15.00
		(0.20)	(0.23)	(0.11)	(0.17)	(0.11)	(0.06)
		14.10	14.95	15.36	15.36	14.52	14.95
v. A.	94.0	20.02	22.84	23.12	23.12	20.30	21.57
		19.88	22.76	22.84	23.07	19.88	21.52
		(0.14)	(0.11)	(0.14)	(0.06)	(0.23)	(0.06)
		19.74	22.56	22.56	22.98	19.46	21.43
	0.94	12.41	12.69	13.40	13.40		
		12.10	12.69	13.28	13.31		
		(0.17)	(−)	(0.06)	(0.06)		
		11.84	12.69	13.25	13.25		
P. H	94.0	26.23	29.32	25.66	25.94	26.09	27.35
		26.06	29.19	25.49	25.80	26.06	27.21
		(0.17)	(0.06)	(0.17)	(0.06)	(0.06)	(0.08)
		25.80	28.75	25.24	25.80	25.94	27.07
	0.94	19.18	20.87			19.18	19.74
		18.92	20.73			18.92	19.66
		(0.11)	(0.03)			(0.11)	(0.11)
		18.75	20.58			18.75	19.46

F. H.	94.0	18.89	18.64 (0.11)	18.47	21.15	20.95 (0.11)	20.87	22.56	22.27 (0.23)	21.71	22.70	22.62 (0.06)	22.56	18.61	18.25 (0.28)	17.77	20.02	19.82 (0.11)	19.74
	0.94	11.56	11.42 (0.14)	11.28	12.97	12.83 (0.06)	12.83							11.84	11.70 (0.14)	11.56	12.13	11.93 (0.14)	11.70
D. G.	94.0	20.30	20.05 (0.25)	19.74	22.56	22.48 (0.11)	22.48	21.86	21.74 (0.06)	21.71	21.86	21.74 (0.06)	21.71						

difference between the fusion frequencies for the singles and fours especially with the higher intensities. At a frequency just about adequate to produce complete fusion of the single spot with the higher intensity, the group of four will still show a coarse flicker. (It is indeed surprising that so clear cut an effect has not been described but that it remained for Adrian and Matthews to indicate the possibility on the excised eye of the eel.)

It has been generally assumed that there is less interaction between adjoining retinal elements in the centre than in the periphery due to the rich supply of horizontal cells in the latter. It is, however, surprising to find the summative power of the periphery so great that areas separated by unstimulated regions of 1.4° of visual angle in diameter (as in arrangement II) should show such marked interaction. Yet, the summation is not complete. If an area as the one covered by the patches in I or II is filled out completely by the same stimulus the fusion frequency will be higher than for the fours. This is shown for two observers in table 2. For one of them(P. H.) the summation is not very far from being complete with arrangement I, for the other one (R. G.) the difference is higher.

TABLE 2

Fixation 10° toward periphery. Intensity 94.0 m.c.

SUBJECT	ARRANGEMENT I	SAME AREA, FILLED OUT COMPLETELY	ARRANGEMENT II
R. G.	24.25	**27.35**	23.27
P. H.	29.19	**30.03**	27.21

These questions will be discussed at some length in a later report of work on quantitative aspects of summation, now in progress.

It is well known that contours are normally blurred in peripheral vision. This is at least partly due to defects of the refractory system at oblique angles of incidence, the irradiation being less marked in the centre. As the amount of irradiation is proportional to the size of the blur-circle and the effect in its essential details can be deduced from well established physical principles Helmholtz (4) was led to suppose that the phenomenon is purely physical. The present experiments, however, show that with the higher intensity and if the stimuli are close to one another (arrangement I), the peripheral flicker spreads over almost all of the interjacent area, even though care be taken to choose a brightness of background against which no scattered light can be distinguished. Coarse peripheral flicker always surpasses the edge of the objects. By giving some information as to what is taking place in the area between the light patches these experiments therefore seem to show that at least in the periphery blurred contours are in part due to a physiological spread of excitation. This is in accord-

ance with the view originally advocated by Plateau (5) which was overthrown on the authority of Helmholtz.

If the stimuli are further apart (arrangement II) four bright flickering centres are seen, the edges of which diffusively infringe upon a steady interjacent area of less brightness. The bright patches seem to be able to influence each other's rate of flicker without causing definite flicker in the area between them. This was still more evident in some experiments with a 100 times brighter background than the one generally used. Thus the spread of the powerful rhythmically waxing and waning excitatory process must be mediated either by ganglion cells in the interjacent area discharging at a lower frequency because of their lower state of excitation (1), (6), or else by direct interconnective fibres joining the four stimulated areas. In view of the little effect visible on the adjacent area compared with the considerable influence on the fusion frequency which remained in spite of trebling the shortest distance between patches, it seems more probable that the spatial summation between distant areas is conveyed along uninterrupted paths between the areas stimulated.

Cajal (9, p. 123) mentions that he has observed in the mammalian eye horizontal branches exceeding 0.8 mm. (over 3° of visual angle), a fact which would seem to presuppose effects of the kind described and also to be in favour of the explanation given to account for them. He also finds the lateral junctions more developed in the higher species of vertebrates.

Discussion. It is interesting to notice that the peripheral retina of the human eye closely resembles the primitive retina of the Conger eel as regards its structure, whereas the human fovea is differently composed. Even the proportion between rods and cones (15–18:1) as given by Chiewitz (7) for various peripheral parts of the human eye is approximately the same as found by Adrian and Matthews (8) in the eye of the Conger vulgaris. The eel's retina also has a complicated structure of interconnected cells, and the receptors outnumber the ganglion cells as in the periphery of the human eye. The similarity of structure between the eel's eye and the periphery of the human retina is not surprising. According to Cajal (9) the histology of the retina shows but small variations between different classes of vertebrates.

Anatomically then the periphery of the human eye seems to be the nearest approach to that prototype of nervous pattern which on evidence derived from work on motoneurones by Sherrington and his co-workers must be expected to display summation *par preference*. The possibility that this interaction should be of the nature of a spread of a photochemical process in the rod and cone layer seems to be definitely ruled out by the work of Adrian and Matthews (1). This view is also strengthened by the striking similarity of the behaviour of motor and sensory neurones more recently pointed out by Adrian and Bronk (10) and by Sherrington (11).

It is worth noting that the appearance of these synaptic reactions in the human eye at least serves the obvious purpose (cf. Adrian and Matthews (1, p. 295)) of being exactly fitted to make the best usage of the contourless, fuzzy light patches thrown upon peripheral parts of the retina. The defects of the refractory system restrict the activity of the photopic periphery to perceiving light. For discrimination of contours there is another organ, the fovea, where each ganglion cell is known to be connected to a single cone and the interconnections between them are less developed.

The basic assumption in this work has been that recording fusion frequencies with the sensory apparatus of vision in simple cases may be closely analogous to the registration of discharges in the optic nerve by means of a galvanometer. The results so far have justified this assumption, though, on the other hand, it is probable that conditions can be found that would alter the fusion frequency as an outcome of processes taking place in higher centres. Thus Sherrington (12) working on binocular flicker in patches joined stereoscopically found even alternating rates of flicker in the two eyes not to influence the fusion point markedly, whereas with very different rates of flicker the fusion frequency altered, probably owing to processes analogous to retinal rivalry. He concluded that under most circumstances the fusion point was determined before the conscious level was reached. Now, the experiments on flicker of Adrian and Matthews (1) show conclusively that the fusion point caused by asynchronism in the discharge as regards intensity and size of the stimulus behaves exactly as the *seen* fusion point in experiments on intermittent vision. At present the best explanation that can be given to account for the occurrence of fusion at certain rates of flicker therefore is that fusion is perceived when the discharging volleys are asynchronous, as they also were found by Adrian and Matthews (8) to be, if steady illumination was employed. Rhythmic waxing and waning with steady illumination, as mentioned above, was found only if the entire retina was illuminated or after application of strychnine.

It has been tacitly assumed that the interaction met with in these experiments is of the nature of a summation. This, of course, has not been directly proved; the inference is mainly based on the fact, pointed out above, that an increase in the intensity of the stimulus is known to raise the fusion frequency. But there remains the possibility that interaction between retinal neurones has an effect upon the fusion frequency *similar* to the one following an increased intensity of the stimulus without necessarily being identical with it. If this interpretation be true, then it certainly would be a rare case of coincidence that would make the reaction time of the discharge of the optic nerve, recorded by Adrian and Matthews, also behave similarly both as regards intensity of stimulus and interaction. This criterion was used by the latter authors, evidently because they

believed a shortening of the reaction time to indicate a physiological effect equivalent to the one following an increased intensity of the stimulus. When the same effect occurred owing to interaction, their natural—though tacit—inference was that the interaction was of the nature of a summation. In a following communication it will be shown that there is an effect of area which cannot be explained on this basis, but apparently conclusive evidence will, however, be added in support of the stated view as to the nature of the interaction described in this paper.

The author is indebted to Dr. D. Bronk for the excellent facilities afforded by his laboratory as well as for valuable critical suggestions.

SUMMARY

In order to obtain information as to whether there is any interaction between distant areas in the human eye, the fusion frequency of four circular test patches, separated by an unstimulated area, has been measured for each of the patches in turn and for all of them together. The four stimuli (see fig. 1), each of a diameter of 1° of visual angle, have been placed symmetrically with their outer edges on an imaginary circle, the diameter of which has been either 3° (arrangement I) or 4° (arrangement II) of visual angle. The test patches have been illuminated at two different intensities, the one 100 times lower than the other. The fixation point has been either in the centre between the stimuli or 10° out towards the periphery.

With central fixation and the higher intensity there is very slight interaction between the stimuli in arrangement I, as shown by the fact that the fusion frequency of all the patches illuminated together is only about $\frac{1}{4}$ of a revolution per second higher than the corresponding value for each of them viewed in turn. With the weaker intensity there is no interaction at all.

With peripheral fixation under similar conditions there is an increase in the fusion frequency of about 2.5 revolutions per second for all the patches together over the value obtained with the single stimuli. The interaction is definite still with arrangement II where the patches are further apart, the fusion frequency for all of them together being about 1.6 revolutions per second higher than the average for the singles. Even if the intensity of the stimuli is diminished 100 times there are still definite signs of interaction left.

The results prove the existence of physiological irradiation in the human eye and thereby give a definite solution of an old problem of vision.

The difference between central and peripheral vision with regard to interaction is correlated with corresponding structural differences in the two parts of the retina.

The general agreement between the outcome of the experiments recorded

above and recent work on interaction in motor and sensory neurones is pointed out.

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THE INFLUENCE OF UREA UPON BLOOD CLOTTING

I. Thrombin Clotting

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Whilst studying various activities of urea, it was observed that this substance exerts a marked influence upon the speed of blood clotting. In low concentrations it retards the clotting of fresh human, horse and rabbit blood, reduces the clotting capacity, on recalcification, of oxalated and citrated horse plasma and interferes with the clotting of oxalated plasma by fresh serum. In still lower concentrations it actually accelerates the process in some of these systems, while relatively high concentrations completely inhibit clotting.

As a basis for the composition of our clotting systems we used the theory of Mills and Mathews (1) (which, in turn, is based upon that of Bordet and Delange), considering normal clotting by thrombin to require the presence of *a*, blood fibrinogen, and *b*, thrombin, which unite directly to form the fibrin of the clot. Thrombin is produced from *a*, serozyme (prothrombin), and *b*, cytozyme, (cephalin from blood platelets), with the intervention of calcium.

1. *The influence of urea upon the clotting of citrated horse plasma.* The plasma used in the following tests was obtained by centrifuging citrated horse blood. In the first series it was employed without further treatment.

In all tables of results, the term "relative clotting time" is the value of the ratio

$$\frac{\text{Clotting time of test mixture}}{\text{Clotting time of control}}$$

The relative clotting times with the lowest urea concentrations, having a value less than 1.00, indicate an acceleration of clotting. The higher concentrations, on the other hand, obviously retard clotting. To produce the initial acceleration, urea must have caused some change in the clotting systems. The most obvious changes would be *a*, a catalytic acceleration in the union of the clotting elements already present . . . . viz., of blood fibrinogen with thrombin to give fibrin, or of cephalin, calcium and prothrombin to form thrombin; or *b*, the liberation of new supplies of one or

more of the primary clotting factors, e.g., blood fibrinogen, cephalin or prothrombin.

2. *The influence of urea on citrated horse plasma, deprived of platelets and tissue fibrinogen by long centrifuging and filtering.* A sample of the plasma used in the first series was centrifuged for about 2 hours and then passed through a Berkefeld "N" filter. The blood fibrinogen of the filtered

TABLE 1

CITRATED HORSE PLASMA	UREA SOLUTION	FINAL UREA CONCENTRATION PER 100 CC.	1 PER CENT $CaCl_2$	CLOTTING TIME	RELATIVE CLOTTING TIME
cc.	*cc.*	*grams*		*minutes*	
1	0.00	0.000	0.2	5.59	1.00
1	0.05	0.185	0.2	4.58	0.82
1	0.05	0.222	0.2	4.88	0.87
1	0.05	0.277	0.2	5.05	0.90
1	0.05	0.370	0.2	5.59	1.00
1	0.05	0.740	0.2	6.75	1.22
1	0.05	1.120	0.2	6.98	1.25
1	0.05	1.480	0.2	6.98	1.25
1	0.05	1.850	0.2	6.98	1.25
1	0.05	2.220	0.2	10.93	1.96
1	0.05	4.250	0.2	50.00	8.94

Note: The control tube contained 0.05 cc. water in place of urea solution.

Note: In this, and in all other experiments reported in this paper, the mixtures stood for 5 minutes in the water bath at 42°C. before addition of calcium.

TABLE 2

CITRATED HORSE PLASMA	UREA SOLUTION	FINAL UREA CONCENTRATION PER 100 CC.	1 PER CENT $CaCl_2$	CLOTTING TIME	RELATIVE CLOTTING TIME
cc.	*cc.*	*grams*		*minutes*	
1	0.00	0.000	0.2	40.00	1.00
1	0.05	0.185	0.2	21.43	0.54
1	0.05	0.370	0.2	20.53	0.51
1	0.05	0.740	0.2	26.91	0.67
1	0.05	1.120	0.2	27.01	0.68
1	0.05	1.480	0.2	27.57	0.69
1	0.05	2.220	0.2	31.83	0.89

plasma, as shown by precipitation by saturated sodium chloride solution, was not appreciably diminished. But 1 cc. of the filtered plasma, mixed with 0.05 cc. distilled water and 0.2 cc. of 1 per cent $CaCl_2$ solution clotted in 40 minutes. The treatment had produced a 7-fold increase in clotting time. This plasma, mixed with urea and tested by the method outlined above, gave the clotting times of table 2.

Here all concentrations of urea accelerate clotting, and the relative acceleration is greater than shown in series 1. But the fact that the clotting time reaches a minimum at about 0.370 gram urea per 100 cc. and then increases, indicates that the final picture is the resultant of at least two processes, one leading to acceleration, the other to retardation of clotting. The latter becomes predominant as the concentration of urea is increased.

It is evident that lower concentrations of urea will produce some change which compensates partially for the clotting factors removed by centrifuging and filtering. This would rather suggest that cephalin at least was liberated by urea, since a fruitful source of cephalin—the platelets—was removed by the treatment given to the plasma. But it is quite possible that another clotting factor—prothrombin—is also liberated from a reserve.

To test the points raised by the results of series 2, a cephalin emulsion (1 per cent cephalin in 0.9 per cent NaCl solution) was added to the centrifuged, filtered plasma. The addition of cephalin caused a great

TABLE 3

HORSE PLASMA	CEPHALIN EMULSION	UREA SOLUTION	FINAL UREA CONCENTRATION PER 100 CC.	1 PER CENT $CaCl_2$	CLOTTING TIME	RELATIVE CLOTTING TIME
cc.	*cc.*	*cc.*	*grams*	*cc.*	*minutes*	
1	0.05	0.00	0.000	0.2	18.28	1.00
1	0.05	0.05	0.177	0.2	6.70	0.37
1	0.05	0.05	0.354	0.2	2.30	0.13
1	0.05	0.05	0.708	0.2	2.05	0.11
1	0.05	0.05	1.062	0.2	1.81	0.10
1	0.05	0.05	1.416	0.2	11.78	0.65
1	0.05	0.05	2.125	0.2	20.55	1.12

acceleration of clotting in the control. It largely, but not completely, replaced the factors removed by centrifuging and filtering. The results of this series are given in table 3.

In this series there is shown once more the resultant of accelerating and retarding factors. But the acceleration is more persistent and relatively greater than in series 2. It is also far greater than in series 1, for while, with 0.185 gram urea per 100 cc. the minimum clotting time of series 1 is 0.82 times that of the control, i.e., 4.59 minutes, the minimum clotting time of series 3 is 13.2/10 or 1.82 minutes, in presence of 1.062 grain urea per 100 cc. In series 3, therefore, at this urea concentration, there has been a change in the clotting system which much more than compensates for the removal of clotting factors by centrifuging and filtering. Moreover this change is so great as to outweigh the ever-present retarding factor, which in series 1 is quite obviously predominant at a urea concentration of 0.74 gram per 100 cc., but in series 3 does not predominate until

there are more than 1.4 gram urea in 100 cc. The fact that this extraordinary acceleration appears not in series 2 where there is little, if any, free cephalin, but in series 3 which contains added cephalin, suggests that urea liberates something other than cephalin—something, such as prothrombin, which combines with cephalin to form an active clotting factor—e.g., thrombin. There cannot be more prothrombin present in series 3 than in series 1 unless it be liberated by urea.

As far as can be deduced from these tests, therefore, the accelerating activity of urea may be ascribed to a possible liberation from a reserve of supplies of clotting factors, the most probable being cephalin and prothrombin. Which part of the blood clotting process does urea retard?

The influence of urea upon the clotting of citrated plasma by preformed thrombin. The process of blood clotting does not consist of one simple chemical reaction, but of a whole series of consecutive and simultaneous reactions. At any given moment thrombin is being produced from its precursors and this thrombin is combining with blood fibrinogen to form fibrin. The fibrin is arranging itself into that peculiar spatial structure which eventually makes the solid clot.

It has been shown above that urea influences blood clotting systems in two ways. It produces both acceleration and retardation, and the trend of the clotting time with increase in urea concentration indicates that both acceleration and retardation are occurring at the same time. The measured clotting time is the resultant of the two. In the first three series of experiments there is evidence that the accelerating activity of urea is exerted upon the reactions leading up to the formation of thrombin, there being, possibly, a liberation of reserves of thrombin precursors. If this be accepted, then the retarding action of urea is possibly exerted upon either the process of formation of fibrin by the union of thrombin and blood fibrinogen, or else upon the orientation of the molecules of fibrin to produce the solid clot.

Serum, freshly pressed from a clot, is very rich in thrombin, and can clot plasma without addition of calcium. In this clotting process the two main reactions will be the union of thrombin with blood fibrinogen and the production of the clot. There will be none of the chemical activity which produces thrombin, unless this is going on in the serum used or unless, in the tests with urea, it be produced by the added urea. This is hardly likely, for the formation of thrombin requires calcium and the only calcium present in a mixture of fresh serum and plasma would be that introduced by the serum, i.e., the excess remaining over from the clotting process by which the serum is produced. But since a minimum quantity of Ca was used to clot the plasma it is unlikely that 0.5 cc. serum added to 1 cc. plasma should leave any excess free Ca. Such a mixture of fresh serum and citrated plasma, therefore, offers a good system in which to study the retarding action of urea.

In a 4th series the plasma-urea mixtures, after standing in the water bath for 5 minutes, were clotted by addition of freshly prepared serum. The original citrated horse plasma was used.

When the predominant processes in the clotting mechanism are the union of blood fibrinogen and thrombin and the orientation of the resulting fibrin

TABLE 4

HORSE PLASMA	UREA SOLUTION	FINAL UREA CONCENTRATION PER 100 CC.	FRESH SERUM	1 PER CENT $CaCl_2$	CLOTTING TIME	RELATIVE CLOTTING TIME
cc.	*cc.*	*grams*	*cc.*	*cc.*	*minutes*	
1	0.00	0.00	0.0	0.2	6.85	5.66
1	0.00	0.00	0.5		1.21	1.00
1	0.05	0.15	0.5		1.42	1.18
1	0.05	0.22	0.5		1.33	1.11
1	0.05	0.30	0.5		1.86	1.54
1	0.05	0.59	0.5		2.01	1.66
1	0.05	0.89	0.5		2.78	2.30
1	0.05	1.19	0.5		7.60	6.28
1	0.05	1.49	0.5		15.37	12.70
1	0.05	1.78	0.5		26.14	21.60
1	0.05	3.56	0.5		97.53	80.60

TABLE 5

HORSE PLASMA	UREA SOLUTION	FINAL UREA CONCENTRATION PER 100 CC.	OLD SERUM	CEPHALIN EMULSION	CLOTTING TIME	RELATIVE CLOTTING TIME
cc.	*cc.*	*grams*	*cc.*	*cc.*	*minutes*	
1	0.00	0.00	0.5	0.00	8.00	9.24
1	0.00	0.00	0.5	0.05	0.87	1.00
1	0.05	0.14	0.5	0.05	0.93	1.07
1	0.05	0.17	0.5	0.05	0.96	1.11
1	0.05	0.22	0.5	0.05	1.02	1.18
1	0.05	0.29	0.5	0.05	1.04	1.20
1	0.05	0.57	0.5	0.05	1.07	1.23
1	0.05	0.86	0.5	0.05	1.35	1.56
1	0.05	1.14	0.5	0.05	6.56	7.57
1	0.05	1.43	0.5	0.05	12.80	14.77
1	0.05	1.72	0.5	0.05	34.21	39.50
1	0.05	3.44	0.5	0.05	121.98	140.85

to give a solid clot, we find that urea shows only a retarding action. There is no evidence of the great acceleration shown in previous clotting systems, all of which involved the production of thrombin from its precursors. It seems probable that our analysis of the previous series was correct.

As a check upon the results of series 4 we made use of the fact that, on standing, serum loses its power of clotting plasma in absence of calcium.

This is supposed to be due to breaking down of thrombin and the binding of the liberated cephalin in such a way as to make it unavailable for the clotting process. Such a serum can, however, be reactivated by addition of cephalin.

Serum as prepared for series 4 was allowed to deteriorate until 0.5 cc. added to 1 cc. of citrated plasma, produced a clot in 8 minutes.

Here again there is shown a retarding action of urea, of the same general order as that shown in series 4. This retarding action is therefore exerted either upon the process of union of thrombin and blood fibrinogen to form fibrin, or upon the formation, from fibrin, of the structure of the clot. Upon which is it exerted? Urea is known to peptise proteins, but usually only in concentrations higher than 3.5 grams per 100 cc.

If it behaves in its usual manner in the process of blood clotting then it is probable that the urea concentrations used in series 4 and 5 are retarding the union of thrombin and blood fibrinogen and not greatly affecting the formation of the clot. It is to be expected that higher urea concentrations will actually peptise the fibrin so that, with sufficient urea present, no clot will form.

In deciding this point, the study has been extended to the action of urea upon fresh, whole blood. At first such blood, treated with urea, seemed to behave quite differently from citrated or oxalated blood. This difference was later found to be only apparent, at least with citrated and oxalated whole blood, and gave interesting information as to the influence of higher concentrations of urea upon blood clotting.

Human blood was drawn directly into sufficient solid urea to make a final concentration of about 10 grams per 100 cc. The blood did not clot. About one hour after drawing, it was diluted with distilled water. A firm clot formed instantly. Washed free from corpuscles, the clot appeared like normal fibrin. Exactly the same result followed with blood drawn from the heart of a rabbit into solid urea to make 10 grams urea per 100 cc.

Blood (850 cc.) was drawn directly from the external jugular vein of a horse into 100 grams of urea powder. The final concentration was therefore about 12 grams per 100 cc. The mixture did not clot spontaneously at any time. Hemolysis was slowly produced. The mixture was not used at once but was placed in the ice box. The vessel containing it was closed only with a cork. No attempts were made to prevent autolysis or bacterial contamination. When examined 3 months later there were no indications of decomposition and no odor of ammonia. A sample of the surface fluid was drawn off with a pipette and placed in a small beaker. It was deep red in color but quite clear. On dropping distilled water into the red liquid, a vortex ring of fibrinous material formed instantly at the point of contact of each water drop. On great dilution there was formed a ring of fibrinous solid, pinkish-white in color, floating in a pale red solution. When dry this solid had all the physical appearances of normal fibrin.

A series of reagents was added to samples of this surface liquid from the blood-urea mixture (about 10 cc. of each reagent to 2 cc. blood mixture). According to results the reagents could be divided into three groups.

GROUP 1 GIVING A FIBRINOUS PRECIPITATE	GROUP 2 PRODUCING TURBIDITY (T) OR A FINE SUSPENSION (S)	GROUP 3 HAVING NO OBVIOUS ACTION
Distilled water	N/5 NaCl (T)	Sat. urea soln.
Ethyl alcohol (95 per cent)	N NaCl (T)	Sat. NH_4CNS soln.
Saturated picric acid solution	2/3 N H_2SO_4 (T)	Oxalated horse plasma
20 per cent trichloracetic acid solution	N/10 $(COOH)_2$ (S)	Fresh serum from oxalated plasma
Sat. ammonium sulphate solution	10 per cent $(NH_4COO)_2$ (S)	Sat. borax soln.
Sat. NaCl solution	30 per cent $(KCOO)_2$ (S)	10 per cent NH_4OH
Sat. boric acid solution	1 per cent $CaCl_2$ (T)	10 per cent CH_3COOH
		Formanide

The turbidity produced with NaCl solution disappeared on diluting about 10 times with distilled water.

The reagents of group 1 suggest that the fibrinous precipitate was of a globulin nature and of an acid reaction. The fact that the precipitate was thready and not flocculent seemed almost conclusive proof that it was blood fibrin.

Another sample of fresh horse blood, drawn into urea to give a final concentration of 10 grams per 100 cc., stood for 24 hours at room temperature. Fibrin was precipitated on dilution.

In both of the experiments with horse blood, the formation of fibrin on dilution was shown only by the upper layer of the blood-urea mixture. Below was a gelatinous mass of corpuscular debris, which gave no fibrinous precipitate with any of the reagents listed above.

Magnesium sulphate blood did not give fibrin with these reagents.

The failure of blood-urea mixtures to clot, even on standing for 3 months in the ice-box, and their immediate clotting on dilution suggested that 10 to 12 grams urea per 100 cc. would allow of the formation of fibrin but not of its orientation to produce the solid structure of the clot. The fibrin was peptised. This view was confirmed by the following experiments on the clotting of recalcified, oxalated *whole* horse blood in the presence of urea.

The sum of A and B above does not deviate far from 9 minutes, the time taken for the clotting of the undiluted control mixture. Therefore, dilution of the control mixture has practically no effect on its clotting time. With the blood-urea mixture, however, it was quite different. Undiluted, this mixture took 26.40 minutes to clot; but in only 15 minutes after the mixture was made it would clot instantaneously on dilution.

We conclude from this that the concentration of urea present, 4.6 grams per 100 cc., allowed of the formation of fibrin, but held it in solution, thus delaying the formation of a solid clot. The urea had, however, also retarded the formation of fibrin, for instantaneous clotting of the urea mixture, on dilution, did not occur until 15 minutes after recalcifying, whereas the control, free of urea, clotted in 9 minutes, either with or without dilution.

TABLE 6

Blood-urea mixture: 10 cc. of whole, oxalated horse blood + 1 cc. urea solution (62 grams per 100 cc.) + 2.5 cc. 1 per cent $CaCl_2$ solution. Stood at 40°C.

Control blood mixture: 10 cc. whole, oxalated horse blood + 1 cc. distilled water + 2.5 cc. 1 per cent $CaCl_2$ solution. Also stood at 40°C.

BLOOD-UREA MIXTURE	CONTROL BLOOD MIXTURE	TIME STOOD BEFORE DILUTION A	DILUTING SOLUTION (0.21 PER CENT $CaCl_2$ SOLUTION,	CLOTTING TIMES AFTER DILUTING			
				Blood-urea mixture		Control mixture	
				B	A + B	C	A + C
cc.	*cc.*	*minutes*	*cc.*	*minutes*	*minutes*	*minutes*	*minutes*
	1	1	1	7.00	8.00		
1		1	1			7.75	8.75
	1	2	1	6.00	8.00		
1		3	1			6.25	8.25
	1	5	1	4.00	9.00		
1		5	1			4.66	9.66
	1	7	1	2.75	9.75		
1		7	1			4.50	11.50
All remainder of control blood mixture clotted, undiluted, in 9 minutes							
1		11	1			2.75	13.75
1		14.5	1			0.25	14.75
1		15.0	1	Instantaneous clotting on 15.00 dilution			
All remainder of blood-urea mixture clotted, undiluted, in 26.40 minutes							

These three actions of urea, which we have described in this article, may go on at the same time in a clotting mixture. The final result, as measured by the clotting time, depends upon the concentration of urea and the composition of the clotting system. In general, low concentrations of urea will accelerate clotting, higher concentratons (up to about 5 grams per 100 cc.) retard it, while still higher (8 grams per 100 cc.) inhibit final clotting by peptising the fibrin formed.

SUMMARY

We conclude, therefore, that urea, added to blood, produces at least three effects:

1. In low concentrations it accelerates clotting by the liberation of reserve supplies of thrombin precursors, i.e., cephalin and prothrombin.

2. In higher concentrations (up to about 5 grams per 100 cc.) it retards clotting by delaying the union of thrombin with blood fibrinogen to form fibrin.

3. In still higher concentrations (8 grams, or more, per 100 cc.) it delays, or completely inhibits, clotting by peptising the fibrin and so preventing its orientation into the solid structure of the clot.

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HYDRION CONCENTRATION AND EDEMA IN PERFUSED HEARTS OF RABBITS[1]

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In a rabbit heart perfused with Ringer-Locke's solution we have a test object without many of the unknown and confusing factors present in the intact organism. Yet it is living in the sense that it will beat regularly and vigorously for hours. Moreover many changes can be made in the perfusing fluid which would be fatal if done to the blood in the normal animal. For these reasons we chose these hearts to begin a study on the causes of edema in living tissue. They are probably more nearly physiologic than most test objects used in similar studies.

The technic of perfusion was the familiar one of Locke and Rosenheim (1907–1908), and two liters of Ringer-Locke's solution were used in each experiment. The hydrostatic head of the solution was 70 cm. and the temperature at which it reached the hearts was kept at nearly 37.5°C. Samples of the fluid were taken just before it reached the heart and also as it dropped away below the heart after going through the coronary circulation. The hydrion concentration of these samples was determined colorimetrically with the use of the appropriate indicators and the color chart which is given in Clark's book (1928).

The hearts were found to beat more vigorously and longer when they were removed from the rabbits as follows: the large arteries and veins in the neck were all severed by a single cut; the thorax was opened and the heart was removed, care being taken not to injure the auricles; and any remaining blood was at once washed out with Ringer solution.

The hearts were rolled gently between the folds of a towel to remove loose water and then quickly weighed. All weighings were done by the same technician who was kept ignorant of the course of the experiments, and are probably accurate to within 0.2 gram.

When the hearts are treated and perfused as outlined, they can be made to beat for eight hours or longer. If they are weighed before and after such a run, the two weights are often about the same. Inspection of the hearts

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during the run, however, shows them to be obviously swollen. Hence it became evident that weighings should be made at intervals during the run. It was found, moreover, that such treatment did not greatly shorten the beating life of the heart and that a time-weight curve could be obtained on a run of many hours. This curve (fig. 1) shows at a glance why we found

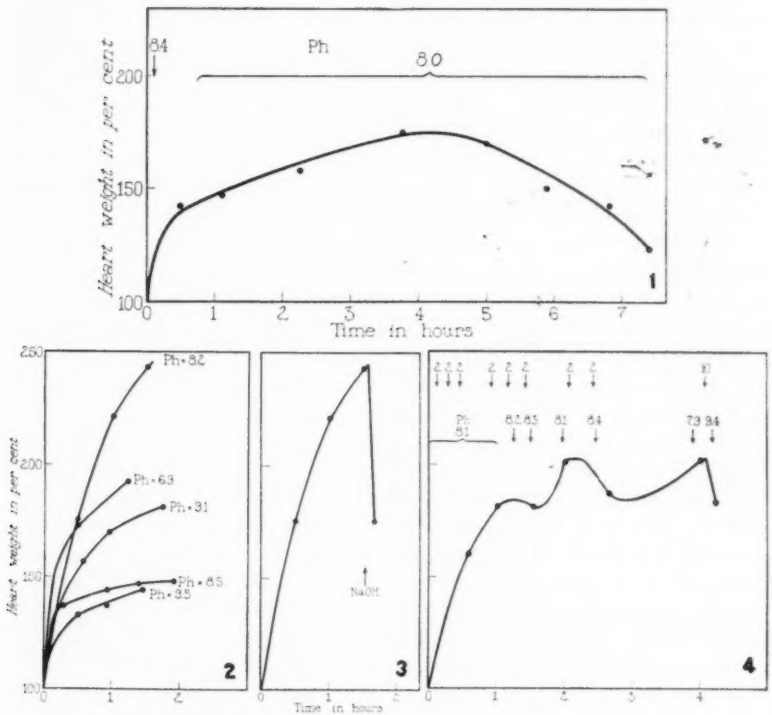

Fig. 1. Standard Ringer-Locke solution.

Fig. 2. Hydrion concentration kept constant during each experiment.

Fig. 3. Hydrion concentration 8.2 at beginning and after addition of sodium hydroxide 9.6.

Fig. 4. Tenth-normal sodium hydroxide added as shown by arrows at top. Numbers = cubic centimeters added.

little increase in weight after eight hours' beating. As the activity of the heart decreases, it loses water. To test out this particular idea further, another heart was allowed to beat, similarly for five hours, at which time it had gained in weight from 5.1 gram to 8.0 gram. This heart was then laid in distilled water. One and a half hours later it weighed only 4.4 grams.

This seems to be similar to syneresis in gels. These facts must not be construed to mean that proteins in the dead heart will not also swell and shrink if conditions are suitably varied. We point out only that during the period when the hearts lose most of their activity there is a tendency to lose water, and unless other more powerful factors intervene they will lose water.

Figure 1 shows also that the heart did gain weight. In the rabbit the water content of the heart muscle had been in equilibrium with the blood. Now it must come into equilibrium with a new fluid. Although approximately isotonic with blood, Ringer-Locke solution is far from being the equal of blood colloidally. Most of the water is in the so-called free state, whereas much of that in the blood is more or less loosely held by the colloids present. We should expect the heart to absorb additional water from such a perfusing fluid. By no change of hydrion concentration were we able to prevent some gain in the weight of these perfused hearts.

The hydrion concentration of the Ringer-Locke solution entering the heart at the beginning of the experiment (fig. 1) was pH 8.4. After about an hour this had changed to pH 8.0, where it remained for the remainder of the experiment. This accidental value is obviously due to an equilibrium between the amount of CO_2 and other acid products coming from the heart and the CO_2 swept out of solution by the oxygen jet pump used to force the liquid into a reservoir at the top of the apparatus.

If we control the hydrion concentration, keeping it constant throughout the experiment, and study the time-weight curves during the first parts of such experiments, we get results such as are represented by the curves in figure 2. It may be seen that if the hydrion concentration is maintained at about pH 8.5, the heart only increases in weight about 50 per cent during the first hour or two of the experiment. Under these conditions the hearts, as a rule, never increase much beyond this amount, however long they beat. But if a little hydrochloric acid is added to the Ringer-Locke solution and its hydrion concentration is raised just 0.3 unit to pH 8.2, the heart increases to more than double its original weight. Still more acid does not cause a still further increase, but as at pH 6.9, there is a distinct drop from the maximal gain. On the alkaline side, the behavior was not so striking, and we found the results not so uniform. However, the general trend is for some extra increase at a hydrion concentration of about pH 9.1. Much greater alkalinity causes the coronary flow to become very small and the heart beats to become feeble. The increase in weight is still less.

There is, then, a region of hydrion concentration for which the increase in weight is minimal. On each side of this region there is increased water-holding power. But this property falls off if the concentrations of acid or alkali are too large. This is, of course, quite parallel to the general behavior of inanimate hydrophilic colloids. However, in this case we really do not know whether the decreases in the amounts of imbibed water at the

higher acid or alkaline concentrations are due to the usual colloid forces or whether these extremes are simply disabling the heart.

These hearts would beat for a considerable time in the presence of Ringer-Locke solution which was much farther on the alkaline side away from pH 7.4, which is normal for blood, than it was possible to go on the acid side and still maintain beating. Since the acid products of muscle work must be removed and the buffer value of Ringer-Locke solution is small, this seems reasonable. It may be noted that a change of only pH 0.3 toward the acid side from pH 8.5 is sufficient to produce great changes in water binding power. Since blood is so much more heavily buffered and the hearts are exposed to it for as long a time as the animal lives, a much smaller change may be effective in vivo. In view of the fact that the almost unvarying constancy of the hydrion concentration of the blood is often urged against Fischer's theory (1921) and other colloid theories of edema, this point is significant. The water content of the heart muscle does not depend directly on the hydrion concentration of the perfusing fluid or of the blood but on the hydrion concentration of the individual muscle cells. These processes are not static equilibrium processes as when a bit of tissue is allowed to stand in a solution. In such a static experiment the buffer value of the solution is not of so great importance as long as the final hydrion concentration is determined. However, in a dynamic experiment, or in life, the muscle units are constantly in contact with fresh incoming liquid of a hydrion concentration different from that which they themselves have. Hence the buffer values of such liquids are of so much more importance in determining the hydrion concentration within the muscle cells.

Certain shifts in hydrion concentration were tried during the experiments to determine whether the amount of edema would also vary. Figure 3 represents such a test. At first the heart was allowed to gain weight under the most favorable conditions of hydrion concentration, about pH 8.2. Then 10 cc. of tenth-normal sodium hydroxide was added which caused the hydrion concentration of the entire Ringer-Locke solution suddenly to shift to pH 9.6. The extra acid which had been combined in the heart was quickly removed and with it much of the extra water.

Another heart (fig. 4) was at first allowed to gain weight under favorable conditions. Two cubic centimeter additions of tenth-normal alkali were made as indicated by the arrows at the top. After the increase was checked, the hydrion concentration was uncontrolled for a while, and it increased and again the heart gained weight. Sodium hydroxide again slightly decreased the hydrion concentration and again there was loss of weight. Once more when uncontrolled the solution drifted toward the acid side and again the heart gained in weight. Again an addition of base was followed by loss of weight.

The opposite effect would be to cause a heart to become a little swollen

because of just the right amount of excess alkali and then to add acid and get loss of weight. When such an experiment was tried a gain in weight, however, was always obtained. The facts that these hearts are usually beating fairly feebly with a small coronary flow and that this beating and flow at once increase when the acid is added may mean that other factors are obscuring the normal colloidal behavior which the inert material would show.

In another experiment the hydrion concentration was started at pH 9.3. The heart beat regularly and its weight increase was the minimal amount. An appropriate addition of acid then raised the hydrion concentration nearly to the region most favorable for edema and the heart at once started to gain weight at a rather uniform rate. If the solution had been maintained much more acid from the beginning, the edema could not reach the maximal possible value. Would a drop in weight occur if still more acid was now added? We found for the first ten minutes after such an addition that the previous rate of increase in weight was checked to some extent, and at the end of the next ten minutes we observed a small drop in weight. Addition of a little base now brought the solution back to about 8.2 and there was a gain of weight. Still more base made the pH 9.2, and we observed a final drop. In every case the weight varied with the shifts in hydrion concentration, just as we might predict.

SUMMARY AND CONCLUSIONS

Perfused rabbit hearts were used to study the water-holding power of living tissue. Many points in common were noted in the behavior of such isolated but regularly working tissue and in the behavior of simple hydrophilic colloids under similar conditions. Although these results cannot be said to bear directly on clinical edema, they are in accord with the hypothesis that the influence of hydrion concentration is one of the factors in such edema.

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CHANGES IN HENS' BLOOD PRODUCED BY A DIET OF SPROUTED SOYBEANS

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It has been reported by Kaupp (9) that gout can be produced in hens by feeding them an abundance of sprouting grain. The disease is caused by the high amino acid content of such seeds. On the other hand, Emmett and Peacock (1) demonstrated that in chicks the presence of urates in the tubules of the kidneys, and at times on the surface of the heart, liver, and spleen, is apparently related to a deficiency of the fat-soluble vitamins. Since the soybean is the only seed thus far investigated which contains the fat-soluble vitamin in liberal quantity (Horneman, 5), it seemed of value to find out whether feeding hens with sprouted soybeans would result in parietal or visceral gout.

Germinated seeds and soybeans in particular contain an abundance of the antiscorbutic factor. It furthermore seemed of importance to find out what would be the influence of such a diet (rich in vitamin C) on the blood clotting capacity. Hess and Fish (cited by Funk, 2) found a normal blood clotting time in Barlow's disease.

METHOD. Seven normal adult hens[1] were fed sprouted soybeans[2] exclusively for a period of forty days. During this period the hens did not show any deviation from normal. Before the experiment was started 1 cc. of blood was taken from the wing's vein and analyzed for uric acid. At the close of the experiment the hens were bled to death from the jugular vein and the blood serum analyzed for glucose, N.P.N., and creatinine by Folin and Wu's method, for blood proteins by Wu and Ling's method, for uric acid (Benedict's method), and for inorganic phosphorus (Briggs' method). Autopsies of these hens were performed and no abnormalities found.

RESULTS. The blood of the hens showed an unusually rapid clotting during the final bleeding. Two-tenths gram per cent of potassium oxalate did not prevent the clotting of the blood during the rapid bleeding from the opened jugular vein.

[1] Nos. 683–687, Barred Plymouth Rocks; nos. 608 and 59, Rhode Island Reds.

[2] The length of the sprouts was 1 to 1.5 inches.

Before the experiment was started the blood contained the following amounts of uric acid (in milligram per 100 cc. of whole blood):

HEN 683	HEN 684	HEN 685	HEN 686	HEN 687	HEN 608	HEN 59
4.21	5.33	4.90	4.42	5.51	5.71	6.15

The analysis of the blood serum (per 100 cc.) at the end of the experiment was:

	HEN 683	HEN 684	HEN 685	HEN 686	HEN 687	HEN 608	HEN 59
Glucose.....	266.6 mgm.	266.6 mgm.	285.4 mgm.	285.4 mgm.	266.6 mgm.	285.4 mgm.	307.1 mgm.
N.P.N.....	31.60 mgm.	28.55 mgm.	33.33 mgm.	30.00 mgm.	31.60 mgm.	33.33 mgm.	35.52 mgm.
Uric acid...	8.22 mgm.	9.33 mgm.	8.75 mgm.	8.75 mgm.	8.75 mgm.	11.66 mgm.	11.66 mgm.
Creatinine..	1.36 mgm.	1.11 mgm.	1.25 mgm.	1.20 mgm.	1.31 mgm.	1.20 mgm.	1.15 mgm.
Inorganic phosphorus ...	3.33 mgm.	2.68 mgm.	4.54 mgm.	3.57 mgm.	3.12 mgm.	3.30 mgm.	3.45 mgm.
Albumin ...	2.06 gm.	2.01 gm.	2.40 gm.	2.01 gm.	1.96 gm.	2.32 gm.	2.48 gm.
Globulin ...	3.42 gm.	2.99 gm.	2.61 gm.	4.07 gm.	3.53 gm.	3.17 gm.	3.31 gm.

DISCUSSION. *Blood clotting.* The rapid clotting of the blood of the experimental hens in the presence of 0.2 gram per cent of potassium oxalate might be attributed to an excess of calcium in the blood, to the presence of 1.65 per cent of lecithin in the dry soybeans or to the antiscorbutic vitamin C. The first cause can be eliminated since Horvath (8) showed that feeding sprouted beans does not affect the blood calcium figures. In order to find out the rôle played by the two remaining possible factors in enhancing blood clotting the following *in vitro* experiments were undertaken: Oxalated blood plasma was diluted with physiological saline solution, a certain amount of $CaCl_2$ solution was added and the clotting time recorded. Parallel experiments were run with the addition of a certain volume of clear extracts of soybean sprouts, sprouted soybean cotyledon, or whole sprouted soybeans (all in saline). With some fluctuations in both directions, the clotting time was on the average unchanged. This experiment seems to indicate that the action of the sprouted soybeans on blood clotting does not occur in a direct way.

Uric acid. This blood constituent shows a marked rise on a diet of sprouted soybeans, referable to their richness in amino acids.[3] The high N.P.N. may also be attributed to the presence of amino acids.

The failure of the hens to develop gout on a sole diet of sprouted soybeans can be attributed to the richness of the latter in fat-soluble vitamins (1), (5).

[3] The percentage of uric acid in the plasma of hens is from 5 to 20 per cent lower than the uric acid content of whole blood determined by Benedict's method.

Since Harding and his collaborators (3), (4) found that in human beings the blood uric acid is raised on high fat diets and since the dry soybean contains around 20 per cent of oil, it seemed of importance to make similar studies on birds. For this purpose ten additional mature Barred Plymouth Rock hens were fed for a period of over two months on various diets containing from 20 to 25 per cent of maize oil. The blood uric acid of these hens did not rise during the feeding of oil, and it can therefore be concluded that the hen's response to this ingredient of the diet differs from that of human beings.

The *serum proteins* in the hens fed sprouted soybeans have an increased globulin: albumin ratio as compared with normal hens (6). This condition may be attributed to the globulin content of the soybean (around 37 per cent of the dry seed) or to the presence of a number of active enzymes in sprouted soybeans (lipase, urease, protease).

Wells (10) found that the relative proportion of albumin and globulin in the serum of milk-fed rabbits did not differ in any significant degree from the proportions observed in rabbits fed on grain and alfalfa. On the other hand, Horvath (6) found that foreign enzymes, after having entered the blood from the intestines, are capable of causing a rise in the globulin fraction of the blood serum. Horvath and Chang (7) have demonstrated that the intestinal wall is permeable for the soybean lipase.

SUMMARY

1. A sole diet of sprouted soybeans for a period of forty days causes the blood of hens to clot in a few minutes, even in the presence of 0.2 gram per cent of potassium oxalate.

2. The uric acid content of the blood serum of such hens shows a definite rise.

3. Feeding sprouted soybeans to hens for forty days does not develop any symptoms of gout, due possibly to the soybeans' richness in fat-soluble vitamins.

4. Feeding sprouted soybeans causes in hens a rise in the globulin: albumin ratio of the blood serum.

5. A high fat ration does not produce in hens any noticeable rise in the blood uric acid.

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A COMPARISON OF THE EFFECTS OF EPINEPHRIN ON CARBOHYDRATE METABOLISM IN THE CAT AND RAT

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In a previous paper on this subject (Eadie, 1929) it was concluded that one and a half hours after the administration of epinephrin to cats under amytal anesthesia the glycogen content of the liver was reduced almost to zero while that of the muscles was essentially unaltered. These findings differed from those of Cori and Cori (1928) who worked with the unanesthetized rat, and it was pointed out that the difference might be due to any one of four causes, viz., the use of an anesthetic, the difference in species, the difference in dosage and the fact that analyses were made at different times after the injection of the drug. It is the purpose of the present paper to report experiments bearing on these possibilities.

Obviously the first thing to be investigated was the effect of the anesthetic. From the experiments reported in the previous paper it appears that the livers of cats starved for 24 hours contain from 1 to 3 per cent of glycogen. Accordingly a preliminary experiment was made with a cat similarly prepared. The dose of epinephrin was, as before, 1 mgm. per kgm. and 1½ hours after this the cat was killed by a blow on the head, the liver immediately removed and 10 gram portions analysed for glycogen. The glycogen content was 0.04 per cent which corresponds closely with the values obtained under anesthesia. From this it appeared possible that one might obtain definite results by comparing normals and epinephrin-treated animals. Two pairs of cats were therefore taken and each pair was treated in an exactly similar way. For a couple of days they were fed the same diet, then starved for 24 hours. To one cat of each pair was given subcutaneously 1 mgm. per kgm. of epinephrin in a 1:1000 solution and to the other an equal volume of physiological saline.[1] After 1½ hours they were killed as before. Analyses were made on 10 gram samples. Results are given in table 1. "Glycogen per cent" throughout this paper means grams of glucose obtainable from the glycogen in 100 grams tissue.

These results are quite definite. The glycogen of the liver decreases, in one case practically to zero. In the muscles in one case we have a loss of

[1] The mode of administration in all experiments in this paper was subcutaneous, and all controls received an equal volume of saline.

glycogen, in the other no change. That is to say there may be a great and prolonged hyperglycemia without alteration in the amount of muscle glycogen. This is quite in accord with the results previously obtained under amytal, and not only confirms them, but also demonstrates that amytal has not vitiated the results of the previous experiments. This excludes the first of the possibilities mentioned above.

We now turn to the second possible cause, the difference in species. Before accepting this one must always reckon with the possibility that some minor technical difference in the carrying-out of the experiments may be the cause of the difference in the results. With this in view it was decided to repeat the experiment with the rat.

The white rat was used. Some of the animals were from the stock colony maintained by Dr. E. V. McCollum, and I am indebted to his department for them; others were from various sources. In the case of the former

TABLE 1

Action of large doses of epinephrin in the cat

CAT NUMBER	TREATMENT	BLOOD SUGAR	GLYCOGEN	
			Liver	Muscle
		mgm. per 100 cc.	*per cent*	*per cent*
2	Epinephrin 1 mgm. per kgm.	—	0.67	0.44
3	Control	—	1.2	0.56
4	Epinephrin 1 mgm. per kgm.	278	0.02	0.30
5	Control	85	0.94	0.32

animals, since the strain was homogeneous, and since they had been kept under standard conditions for many generations, it was thought sufficient to pick rats of approximately the same age; in the case of the latter only litter mates were used, and of these any that differed markedly in weight from the others were rejected.

In the first experiment (table 2, expt. 1) the dose used was 0.1 mgm. per kgm. The animals were killed by decapitation an hour after the injection. The whole liver was used for analysis, and muscle samples from the leg of about 5 grams, care being always taken to use corresponding muscles.

In this experiment hyperglycemia is not marked. There is, however, a definite increase in the liver glycogen and an equally definite decrease in that of the muscle. This is in full agreement with the results reported by Cori and Cori, and therefore indicates that my methods of experimentation were sufficiently similar to theirs. This increase of liver glycogen may be termed the positive phase, and a decrease the negative phase. It was suggested previously that the positive phase might be a late phenomenon secondary to a negative phase. We know that the positive phase is present

three hours after injection from the work of Cori and Cori; from these experiments we see that it is already present one hour after. It was then decided to decrease the interval to 20 minutes in the hope that the negative phase might be present then. This was done in experiment 2 (table 2). It will be seen here that the liver still shows the positive phase. There is only a slight hyperglycemia. The results with the muscle are less clear since there is a large variation in the glycogen content of the controls. These rats, however, were from the same source as the preceding (Dr.

TABLE 2

Action of epinephrin on the rat

EXPERIMENT NUMBER	DOSE	TIME AFTER INJECTION	EPINEPHRIN			CONTROLS		
			Blood	Liver	Muscle	Blood	Liver	Muscle
	mgm. per kgm.	*minutes*	*mgm. per 100 cc.*	*per cent*	*per cent*	*mgm. per 100 cc.*	*per cent*	*per cent*
1	0.1	60	103	3.0	0.28		1.9	0.46
			126	3.0	0.24		1.5	0.46
			128	2.9	0.24	96	2.1	0.50
2	0.1	20	166	3.2	0.28	149	2.0	0.24
			174	2.8	0.20	134	1.1	0.70
3	0.1	10	120	3.9	0.47	120	4.3	0.36
			124	5.3	0.45	128	5.2	0.49
4	1.0	20	184	0.53	0.27	116	0.33	0.39
			261	0.49	0.23	114	0.54	0.45
5	1.0	20	208	2.5	0.42	129	5.7	0.31
			224	2.4	0.35	133	4.5	0.29
6	1.0	20	224	5.2	0.34	126	6.1	0.42
			212	2.5	0.52	133	4.4	0.25
7	1.0	90	310	1.3	0.16	116	1.8	0.40
			350	0.43	0.22			

McCollum), and both sets had been on the same diet since birth, and both were very nearly of the same age; it is therefore probable that no great error would be made in taking the controls of the previous set, especially since the liver glycogens both in the controls and the epinephrin-treated animals, and the muscle glycogens in the latter are all within a close range in both sets of rats. If one accepts this, there is a decrease in muscle glycogen; otherwise no conclusion can be drawn.

It was regarded as highly improbable, but not altogether impossible that a negative phase in the liver might be present at a still earlier period, and

to exclude this another experiment was made in which rats were killed 10 minutes after injection (expt. 3, table 2). It is obvious from inspection of these figures that no change has yet been produced by the drug: there is no hyperglycemia, and no definite change in either muscle or liver glycogen.

From this series of experiments, then, it appears that the dose of 0.1 mgm. per kgm. of epinephrin in the rat produces a mild hyperglycemia, and at the same time a decrease of muscle glycogen and an increase in that of the liver.

It is, however, hardly fair to compare these experiments with those on the cat described above, since the dose in the case of the latter was ten times as great as in the former. The larger dose was therefore tried in the rat. In the first experiment the rats were killed 20 minutes after injection (expts. 4, 5 and 6, table 2). The results here show a certain variation, but differ essentially from those obtained with the lower dosage. In all there is a marked hyperglycemia. In experiment 4 the rats had been starved for a longer interval, and the initial glycogen values in the liver were low. Here epinephrin seems to have produced no change in the liver and a decrease in the muscle. In experiment 5 where the initial amounts of glycogen in the liver were high, the liver glycogen decreases. While no definite change can be demonstrated in the muscle there is certainly no decrease. In experiment 6 one treated rat shows no definite change in the liver and the other a decrease. The muscle shows no definite change either way. It then seemed possible that if these processes were allowed to continue longer, as in the cat, a more definite result might be obtained. Accordingly in experiment 7 (table 2) an interval of 90 minutes was allowed to elapse before killing the animals, but there was little difference in the results. In one rat the liver glycogen was very markedly decreased, in the other this was not so marked. Muscle glycogen appears to be decreased. The hyperglycemia is very marked.

Summing up, then, the results with the larger dose in rats, we may say that the liver glycogen never shows an increase, but always a greater or smaller decrease. Muscle glycogen does not usually show a decrease in 20 minutes but may do so later. This demonstrates that the dosage also plays an important rôle in determining the results in the rat and makes it necessary to investigate this factor also in the cat.

Experiments in the cat using a dose of 0.1 mgm. per kgm. are given in table 3. Hyperglycemia is not marked. In experiment 1 the cats had been starved for a longer period than in experiment 2, and the initial liver glycogens were lower. In both experiments, however, the liver glycogen decreases. The muscle glycogen shows no definite or uniform change. This is essentially what was found with the larger dosage, and here the effect of alteration of dose is not seen.

We may therefore accept the species-difference as the main cause of the differences in results obtained by Cori and Cori and myself, noting at the same time that by using enormous doses in the rat, one may alter the character of the results. The difference in time relations does not seem to have had any effect.

Discussion. Before discussing the results reported in this paper a few words are necessary in explanation of the conclusions advanced in the previous paper in view of the criticisms of Cori and Cori (1929). The experimental facts are as follows: two cats under amytal anesthesia were given doses of epinephrin; samples of muscle (and liver) were taken before and after. The cats showed an approximately equal degree of hyperglycemia; in one cat the muscle glycogen changed from 1.3 per cent to 1.0 per cent; in the other from 0.93 to 0.90 per cent. In other words, the hyperglycemia was accompanied in one case by a certain drop in the muscle

TABLE 3

Action of small doses of epinephrin in the cat

CAT NUMBER	TREATMENT	BLOOD SUGAR	GLYCOGEN	
			Liver	Muscle
		mgm. per 100 cc.	*per cent*	*per cent*
6	Epinephrin 0.1 mgm. per kgm.	132	0.22	0.16
7	Control	75	0.54	0.16
8	Epinephrin 0.1 mgm. per kgm.	124	0.85	0.40
9	Epinephrin 0.1 mgm. per kgm.	176	0.63	0.39
10	Control	96	3.9	0.33
11	Control	91	5.2	0.45

glycogen; in the other, which in every other feature was exactly the same, there was, as Cori and Cori admit, no change. They, however, attach much more importance to the first case, while I think that emphasis should be laid on the second, and for this reason, that if mobilisation of muscle glycogen be an essential part of the hyperglycemia produced by epinephrin, one should never occur without the other. The fact that there has been marked hyperglycemia (e.g., cats 4 and 5) with no definite change in the muscle, seems to me to indicate that mobilisation of muscle glycogen depends on other, as yet unrecognized, factors. One may conclude, therefore, from these experiments and those described in this paper, that in the cat the action of epinephrin does not necessarily nor indeed frequently lead to a diminution of the glycogen of the muscles.

In the rat lower doses always produce a decrease of muscle glycogen, but this may be absent with higher doses. The cause of the variation with high doses appears to be related to the initial value for liver glycogen, since when

it is low the muscle shows a decrease. From this one might conclude that there has been a transport of glycogen from the liver, but there are many difficulties in this view which will be discussed later.

Turning now to the liver, we have seen that, in the cat, the effect of epinephrin during the first hour or so is invariably, with both dosages, a reduction in its quantity of glycogen. This corresponds closely with what Sahyun and Luck (1929) have found to occur in the rabbit during this period. This negative phase, they find, is succeeded by a positive phase lasting up to the 18th hour, the stimulus for which may be either the epinephrin directly or the depletion of the liver glycogen. There is no evidence in these experiments for transport of glycogen, for in the first phase there is a concomitant decrease in both liver and muscles, and in the second there is no further decrease in the muscles at a time when there is an increase in the liver. It seems more probable that this increase is due to a synthesis, perhaps from lactic acid although quantitative data, sufficient to decide this point, are lacking.

In the rat, on the other hand, we find with moderate doses an increase of liver glycogen, with larger doses sometimes no change and sometimes a decrease. Since the increase occurs in the earliest stages, it is doubtful whether it is due to the same causes which produce the increase in the rabbit. We may explain these various results by assuming that there exist in the liver two processes, of synthesis and hydrolysis of glycogen, both directly stimulated by epinephrin, but to varying degrees depending on the dosage. This synthetic process which was first brought into prominence in this connection by Cori and Cori does not occur to any extent in the cat or rabbit, at least in the early stages.

Although this is perhaps the simplest explanation, we should not forget another possibility, viz., that the large dose of epinephrin acts in a toxic manner on the liver, thus preventing synthesis. Unfortunately we know almost nothing about such toxic actions of epinephrin, nor do we know how fast it enters the blood stream when injected subcutaneously. Our only criterion is at present the degree of hyperglycemia produced. Judging by this, 0.1 mgm. per kgm. which produces only slight degrees of hyperglycemia (20 to 30 mgm. per 100 cc., well below the range of glycosuria) is not greatly removed from the physiological range. The larger dose, however, is far above this range. Let us assume that the larger doses act in a toxic way upon synthesis. This, while it accounts for the happenings in the liver, does not offer any simple reason for the comparative constancy in the muscle; one does not see, on the one hand, why a toxic process as such should prevent a loss of glycogen, nor, on the other hand, if one assumes that the toxic action is confined to the liver, why inhibition of synthesis there should prevent a peripheral loss even if the synthetic process makes use of lactic acid derived from the muscles. Moreover, as we shall see

below, if one accounts for the lack of fall in the muscles as being due to extra supplies from the liver, one must assume the presence there of an active synthesis. We may, of course, choose our hypothesis so as to attribute to the toxic action only increased hydrolysis of glycogen in the liver. In this case we must decide whether the small doses which lower the liver glycogen in the cat are also toxic in that animal. All these alternatives only involve us in greater difficulties, and can only be resolved by further experiment. Until we know something definite about the toxic action of epinephrin, it appears simpler to retain our first hypothesis of two normal processes unequally affected by increasing doses.

There is a certain amount of evidence in the rat for the transfer of glycogen from the liver to the muscles as mentioned above, viz., with large doses when there is a good deal of glycogen in the liver one fails to see the fall of muscle glycogen. Himwich, Koskoff and Nahum (1930) attribute to this cause the constancy of muscle glycogen under amytal anesthesia. While this is very probably true, simple calculations will show how impossible it is to draw up a balance sheet. The bulk of the muscle is so much greater than that of the liver that if we were to account for it merely by the loss of glycogen from the liver, the changes there necessary would be enormous. As a matter of fact, we find only very small changes in the liver, which are quite inadequate. On the other hand, it is probable that glycogen synthesis is occurring in the liver to an unknown degree, and we may, if we choose, assume that this is sufficient to account for it. In this case, however, we must introduce secondary assumptions to explain why this synthesis is more active when the liver stores are full of glycogen, a state of affairs which is rather improbable. In the absence of experimental evidence it is useless to pursue this argument further, and we can only note an apparent relation between the glycogen content of the liver and its constancy in the muscle. In passing it may be remarked that so far no mechanism has been discovered which increases the muscle glycogen above normal at the expense of the liver (unless this has occurred in experiment 5, table 2, but as this might be due to the uncertainties of the method, it cannot be used as evidence). Why this is so is not known; but it probably indicates the presence of a regulating mechanism defining the upper limit for glycogen content of the muscle.

There is no doubt, however, that the process in the three animals studied shows many points of difference and is undoubtedly more complicated than has hitherto been thought to be the case. The difference in action in different species has of course been noted with other things, e.g., the action of pituitrin on blood pressure in fowls and mammals. For the present it seems advisable to exercise greater caution in transferring results from one species to another.

SUMMARY

1. Amytal does not alter essentially the effect of epinephrin on muscle and liver glycogen.

2. A small dose of epinephrin in the rat is followed by an increase of glycogen in the liver and a decrease in the muscle; and a large dose by a decrease in the liver and often a decrease in the muscle.

3. In the cat both the large and the small dose are followed by a decrease of glycogen in the liver; in the muscles there is usually no change.

4. The difference between these two species is not due to difference in dosage or in time of sampling, but is thought to be a definite species difference.

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THE PSYCHOGALVANIC REFLEX AS RELATED TO THE POLARIZATION-CAPACITY OF THE SKIN[1]

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Although Veraguth gave the name "psychogalvanic reflex" to this phenomenon in 1906, it had been noticed previously and has been studied by a great many investigators (Landis and DeWick).

Two phenomena are measured electrically. Tarchanoff measured the bio-electric current of the skin but Fere and most workers have measured the electric impedance. As a matter of fact, these two phenomena depend upon the same change in the cells. It has been shown by the study of bio-electric currents and electric conductivity of cells in general that they both depend on a change in the cell surface and, although the chemistry of the process is not known, the physics of it has been worked out. It has been shown repeatedly that the resting cell is electro-negative on the inside and, therefore, has an electric-double layer on the surface, since the electro-negative interior attracts a superabundance of electro-positive charges to the neighborhood of the surface. When a cell is ruptured or stimulated at one point, electro-negative charges come out at that point and the double-layer is destroyed. Although it is not known what ions these electrons are attached to, it has been established that electrons come out of the cell at the point of rupture or stimulation. It is, therefore, safe to assume that in the resting cell these negative charges are held in by forces other than the general electric field. The simplest assumption is that the negative charges are the charges of ions to which the cell surface is impermeable, and that rupture of the cell, or even stimulation, increases the permeability of the surface. It has been shown by experiments of Höber, Philippson, Fricke, McClendon and others, that ions are free to move in the interior of the cell.

Measurements of the thickness of the impermeable layer on the cell surface depend on certain assumptions, the chief of which is its dielectric constant, but with all possible assumptions of dielectric constant it may be shown that this layer is very thin on the surface of some resting cells; in fact, it is not greater in thickness than the length of a fatty-acid molecule. Since it has been shown by Langmuir and others that fatty-acid

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molecules may orient on a surface with their long axes perpendicular to the surface, and since combinations of lipoid and protein have been known since the work of Hoppe-Seyler, it seems probable that the cell surface consists of oriented fatty-acid molecules, bound up, perhaps, in the form of lecithin and cholesterol-ester, and attached by chemical or other bonds to the protein beneath. Hansteen-Cranner was able to wash off some of the lipoid from the surface of cells without destroying the cells, and it was shown by Overton that lipoid-soluble substances pass readily into cells. But since the cell is a water-phase and the medium is a water-phase, it would be impossible to maintain a thin, fluid, non-aqueous phase on the cell surface without some special mechanism of holding it there, and the lecithin-protein combination seems the most probable mechanism. In the eggs of fish and amphibians there are crystals of lipoid-protein, which are optically void and are not two-phase systems. It is only necessary to assume that a lipoid-protein exists on the surface in such a way that lecithin forms a continuous layer on the surface, in order to give an imaginative picture of the impermeable surface layer (that has been called the "plasma membrane" by biologists).

Rupture of the surface layer of a cell allows negative ions to come out of the cell but the corresponding positive ions had already come out of the non-ruptured plasma membrane and, therefore, the plasma membrane must be permeable to some positive ions at all times. We would immediately think of the smallest, most rapidly diffusing ions, in others words, hydrogen ions, as the ones always able to pass. It is not so easy to guess the negative ions. They should be of substances that have been determined chemically and probably not carbonic acid anions (because a cell is easily permeable to CO_2, and it would be hydrolyzed on the outside of the cell, and thus produce equal concentrations of carbonic-acid anions outside as inside). It seems more probable that they are lactic acid anions, since lactic acid is produced by all cells, and the anions are larger than CO_2, and it is less likely that the plasma membrane is permeable to them.

A difficult problem for the imagination is to picture the change in the plasma membrane that takes place on stimulation and allows the anions to come out, and yet, in a few seconds is reversed, restoring the impermeability of the plasma membrane. We might assume that stimulation consists in knocking down the nine-pin arrangement of fatty-acid molecules, thus leading to a disorderly arrangement with interstices large enough for the passage of the anions. With the reversal of this, there is a restoration of the orderly arrangement. It has been shown that electric stimulation consists in the sudden approximation of a cathode to the plasma membrane, and if the distribution of electric charges on the fatty-acid molecules is sufficiently irregular, this sudden change in potential

might exert considerable force on them. The re-ordering of their arrangement need not be any more difficult to imagine than the formation of a film of fatty acid on the surface of a water phase, resulting in the orderly arrangement of the molecules.

The question whether the whole skin is involved in the psychogalvanic reflex or what parts are concerned, has been very much debated. It has been shown that the stimulus comes down sympathetic nerves and that these same nerves upon stimulation cause a contraction of the blood vessels and a secretion of sweat. The blood vessels have very little to do with the electric resistance of the skin. The blood corpuscles are highly resistant but the blood plasma is of such low resistance that the whole blood has only moderate resistance. It is necessary to have a layer of cells packed so closely that very few ions can pass between them in order to have a very highly resistant structure, like the skin. The resistant layer is the Malpighian layer which extends over the surface and is reflected inward to form the sweat glands. According to Ebbecke, the general Malpighian layer can be stimulated only by local irritants and not through these sympathetic nerves, so that (by exclusion) the sweat glands must be the seat of the psychogalvanic phenomena.

There has been some argument about the prefix "psycho" as applied to the psychogalvanic reflex but it is not a simple spinal reflex. The psychogalvanic reflex is not due to the stimulus alone but to awareness of the stimulus or attention of the subject (and disappears when he is bored). It may be produced by a painful stimulus and be repeated when the subject is told that the stimulus is to be applied and yet is not applied. It may be the result of auditory, optical, olfactory, heat, cold or touch stimuli. The psychogalvanic reflex is very prominent is emotional states; in fact, when these lead to visible sweating and blanching of the skin there is a very marked reflex. Under primitive conditions both the sweating and vascular reflexes might be imagined to be concerned with preparedness for fighting. The sweating might provide for an immediate loss of the excess heat to prepare for muscular exertion and the contraction of the cutaneous vessels cause filling of the heart and greater cardiac efficiency.

The fact that the reflex might be demonstrated in some persons with a continuous flow of sweat, does not prove that the sweat glands are not concerned, because this continuous flow of sweat might not involve all of the sweat glands or all of the cells of each sweat gland, and until we know the mechanism of the pathological sweating, we should not use this as an argument against the theory that the sweat glands are involved in the reflex. It has been shown by Leva that the reflex is distributed in different parts of the skin similar to the sweat glands.

The best technique in the study of the psychogalvanic reflex, so far as the electric measurements are concerned, has been developed by Gilde-

meister, and the present paper concerns itself mainly with the refinement of Gildemeister's technique. Gildemeister distinguished the difference between "ohmic" resistance of the body and the polarization-capacity of the skin.

We have built a Wheatstone bridge (fig. 1) of equal ratio arms which, we believe, is superior to any bridge previously used for high-frequency

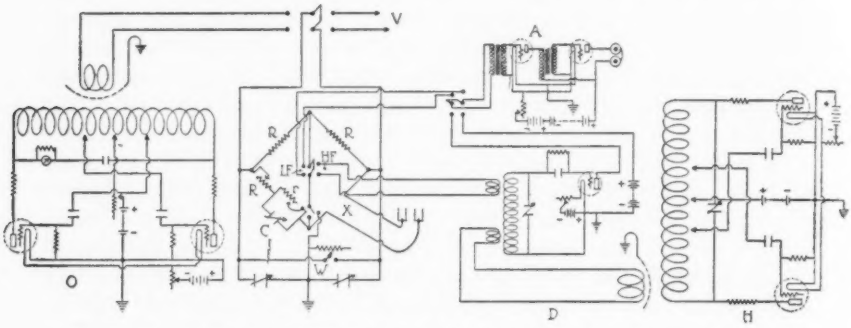

Fig. 1. Circuit diagram of Wheatstone bridge for the study of the psychogalvanic reflex. The high-frequency oscillator is shown at *O* and may be connected or disconnected by means of a double pole double-throw switch, which is also used for the Vreeland oscillator not shown in the figure, but the wires to it are indicated at *V*. The electric circuit is made symmetrical by means of a Wagner ground, indicated at *W*. The equal ratio arms of the bridge are shown at *R*,*R* and in one arm the unknown is connected at *X*, which is led outside the bridge shield and connected with two electrodes, in which the fingers may be immersed. The remaining arm of the bridge contains the variable series-resistance *R*, variable capacity *C* and the variable parallel-resistance *r*. The detector circuit contains a double-throw-double-pole switch, which may be thrown into the amplifier for low frequency, indicated by *LF*, or thrown into the electron tube detector, indicated by *HF*. The detector *D* is connected by inductance with the double-throw switch of the detector circuit of the bridge on the one hand and the heterodyne oscillator *H* on the other. The amplifier *A* may be connected by the double-throw-double-pole switch either directly to the bridge through *LF* or directly to the detector. The oscillator *O* consists of a twin circuit in which the grid of each tube is connected to the oscillating circuit of the other. The heterodyne *H* is of similar construction. The detector *D* contains a single oscillating circuit. The amplifier *A* is of two stages and, owing to the impedance of the transformer, it is most efficient at 1000 cycles.

electric currents. We have made measurements on the plasma membrane of red blood corpuscles and shown we could distinguish the resistance of the cell interior, *R*, from the resistance of the plasma membrane, *r*, and the capacity of the plasma membrane, *C*.

When a high-frequency electric current is passed through the cell the plasma membrane offers very little impedance to the current. More than 99 per cent of the impedance is the true ohmic resistance of the cell

interior. The plasma-membrane, then, acts as a condenser of large capacity in relation to the high-frequency current and its impedance is very low.

With this Wheatstone bridge, we could study a mass of red blood corpuscles (packed down by centrifuging at 20,000 revolutions a minute in a vacuum so as to remove the blood serum) by measuring the impedance at a million and a half cycles per second, and by balancing this in the bridge by a resistance, R, then placing in series a second unit (consisting of a capacity, C, in parallel with a resistance, r) and with an audio-frequency current we could determine the value of the capacity and resistance of the plasma membrane.

It only remains to apply this method to the skin. One difficulty arises from the fact that the part of the skin being measured could not be cut off and put in the shielded part of the apparatus but this difficulty was overcome by trial and error so that some measurements which appear to be accurate were made.

Another difficulty arises in marking off a constant area of the skin. This has been done by Densham and Wells by painting part of the skin with collodion. We showed that this is a very poor method and probably accounts for the erroneous conclusions of Densham and Wells that the change in impedance is due to stretching following vaso-constriction. In the first place the collodion is permeable to the electric current. In the second place its resistance is changed by stretching. Stretching not only changes its thickness but tends to lift it up from the skin or cracks it in such a way that it does not come back to its original value after being released. This applies not only to pure collodion but to various mixtures of collodion and castor oil (added to make collodion pliable). Densham and Wells assume that stretching the skin changes its resistance but this change is evidently due to stretching the collodion and not the skin. We found that if vaseline is used to mark out the area of the skin, these effects disappear, so the conclusions of Densham-Wells that the psychogalvanic reflex is due to contraction of the capillaries, which in turn stretches the skin, is erroneous. The capillaries contract as the result of a stimulus coming down the same sympathetic nerves as the stimulus to the sweat glands and is merely a side reaction.

Our method is to coat two fingers of the same hand, except the terminal joints, with vaseline and immerse them in separate electrode vessels, shown in figure 2. The 1 per cent NaCl solution in the electrode vessels was kept at approximately constant temperature. The electrode vessels were connected with the bridge.

The first experiments show that the impedance to the high-frequency current does not change during the reflex. The arrangement was used for simultaneous measurements of the direct-current-impedance and the

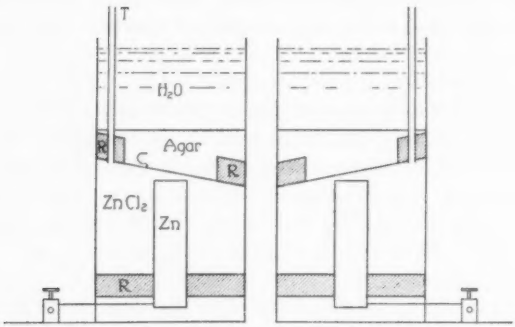

Fig. 2. A pair of electrodes for leading off from the bridge. Each electrode consists of a glass cylinder, the lower end of which is closed with a rubber stopper R through which passes an amalgamated zinc rod, Zn, connected with a binding post by means of a copper wire soldered to its lower end. The zinc rod is immersed in a solution of zinc chloride, $ZnCl_2$. The top of the zinc chloride solution is pushed by a cellophane diaphram C attached to a rubber ring R. The air bubbles escape through tube T. On top of the cellophane diaphram a hot solution of agar in 1 per cent NaCl is allowed to flow, which gels on cooling. On top of the agar is poured a 1 per cent solution of NaCl in water, H_2O.

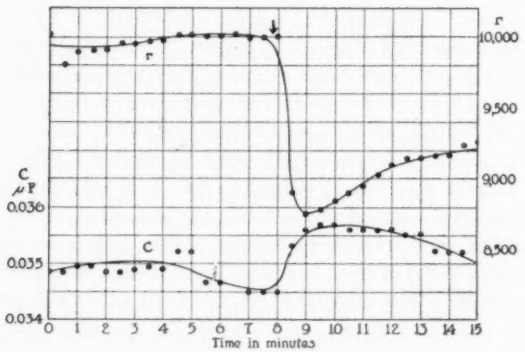

Fig. 3. Graph of the effect of stimulating another part of the body by an electric stimulus on the resistance and capacity of the plasma membrane and the sweat glands in the skin of the finger. In this case the series resistance of the fingers R was 900 ohms. Before stimulation the resistance of the plasma membrane, r, remained about 10,000 ohms but on stimulation, indicated by the arrow dropped to 8,700 ohms. C remained about 0.035 microfarad, although the measurements of it were not so accurate as those of R and the individual determinations and showed a wider variation, indicated by the black dots. On stimulation there was a distinct increase in C up to 0.0357 microfarad.

impedance to a current of a million and a half cycles. The average of the results shows that when the impedance changed 13.7 per cent to direct current the impedance to a million-and-a-half-cycle-current changed 0.93 per cent. For practical purposes, then, we may assume that the high-frequency impedance does not change during the reflex. The D. C. apparatus was then removed, the electrodes remaining connected to the bridge and measurements made in quick succession by means of the high-frequency current and then by a current of 1000 cycles per second. By means of the high-frequency current the "ohmic" resistance of the fingers, R, was determined and then fixed, and by means of the 1000 cycle current, r and C were determined in the resting skin, as shown in figure 3. In this case R was 900 ohms and C and r remained almost constant for $7\frac{1}{2}$ minutes. At this time a stimulus was applied to the other hand by means of an induction coil. A marked reflex was shown and there was a very sudden drop in r from 10,000 ohms to 8,800 ohms. At the same time there was an increase in C from about 0.035 microfarad to 0.0357 microfarad. This increase in C does not necessarily mean a thinning of the plasma membrane but may be merely a thinning of the Helmholtz-double-layer due to the movements of ions.

SUMMARY

The psychogalvanic reflex is due to a change in polarization-capacity of the plasma-membranes of the cells lining the sweat glands in the skin. The contraction of the blood capillaries is a reaction following an impulse traveling along the same sympathetic nerve as the impulse to the sweat glands.

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THE GROWTH AND DEVELOPMENT OF CHICKS AS INFLUENCED BY SOLAR IRRADIATION OF LONG VISIBLE AND ULTRAVIOLET WAVELENGTHS, RESPECTIVELY, WITH AND WITHOUT SUPPLEMENTARY IRRADIATION OF VARIOUS TYPES

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In 1928, we reported the results of our investigations on the effects of selective solar irradiation on the growth and development of chicks (3) and on the parathyroid glands of chicks (2). Eight groups of chicks were used. Two groups were housed behind quartz-containing glass (vitaglass), ordinary window glass, amber glass (Pittsburgh no. 48), and blue glass (Pittsburgh no. 56), respectively. The standard Wisconsin all-mash ration was fed to one group under each type of glass filter, respectively, and to the other group under the specified selective filter the same ration to which 2 per cent (by weight) of Squibb's cod liver oil had been added. We concluded that there was evidence to support the statements that 1, cod liver oil (2 per cent by weight added to a diet adequate in all ingredients except vitamine D) is able to induce normal growth and development irrespective of the presence or absence of any portion of either ultraviolet or visible solar energy, and 2, normal growth and development of chicks, in the absence of cod liver oil, are dependent on both the ultraviolet and visible portions of solar radiation, all other factors remaining constant so far as is known.

In order to put to further test the statement that normal growth and development are dependent on both the ultraviolet and longer visible portions of the solar spectrum, we carried on, in the summer of 1928, a series of experiments similar to those previously reported by us, but we housed the chicks (groups 1 to 6 inclusive) behind 8 square feet of amber cathedral glass (Pittsburgh no. 48) 3 mm. thick, which transmits only the long wavelengths of visible solar irradiation (practically from 500 millimicrons to the extreme red end of the visible spectrum), and supplemented this everyday selective solar irradiation of chicks with the longer visible wavelengths by giving daily periods of irradiation with the air-cooled quartz-mercury lamp

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(Victor x-ray, operated at 90 volts and at a distance of 50 cm., the incident ultraviolet energy (1) being 575 ergs each second for each square millimeter of receiving surface). We also housed two groups (groups 7 and 8) of chicks behind 8 square feet of purple corex filter (Corning Glass Company G 986 A, 7 to 8 mm. thick and unpolished) which transmits hardly any visible light but does transmit a goodly portion of the ultraviolet light between 400 and 270 millimicrons (fig. 1), and in one group (group 8) we supplemented this irradiation through corex glass by giving daily fifteen-minute periods of irradiation with outdoor sunlight filtered through the amber glass (Pittsburgh number 48). By these methods of procedure we supplemented, in certain groups, the daily reception of the energy contained in the longer wavelength portion of visible solar irradiation (amber

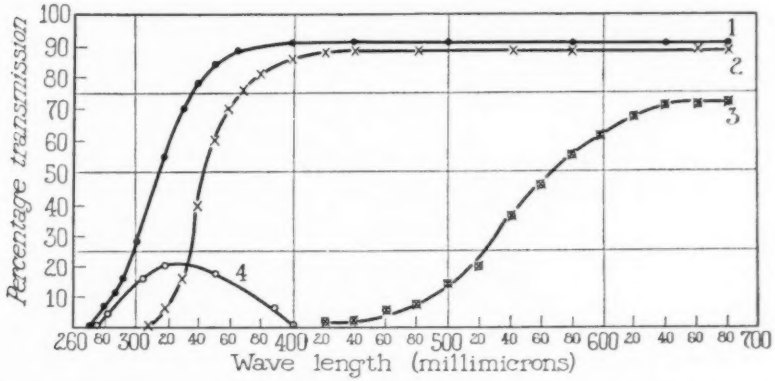

Fig. 1. Spectrophotometric determinations of the percentage transmission of various wavelengths by vitaglass (curve *1*), ordinary window glass (curve *2*), amber glass (Pittsburgh, no. 48, curve *3*) and corex (purple, Corning G 986 A, curve *4*).

glass) with the ultraviolet portion of the irradiation from an air-cooled quartz-mercury arc and, in certain other groups, we supplemented the daily reception of a considerable percentage of the ultraviolet portion (290 to 400 millimicrons) only of sunlight through the use of purple corex glass with exposure to solar irradiation of the longer visible character. The ration fed in all instances was the Wisconsin all-mash ration consisting of 80 pounds yellow corn meal, 20 pounds shorts, 5 pounds bone meal, 5 pounds limestone grit, and 1 pound salt; skimmed milk was given freely without water the first two weeks.

EXPERIMENTAL DATA. The scheme of our investigations, as regards the types of filters, periods of irradiation with an air-cooled quartz-mercury lamp, parts of the bodies of chicks exposed, and so forth, is given in table 1.

The growth and development of the chicks was estimated by obtaining the average weight of the same group of ten chicks from each of the eight compartments, respectively. The results for the one hundred fifty days of the experiments are shown in the curves of figure 2. Data relative to weights (ounces) of the selected groups in the various compartments, under the various conditions of irradiation specified, at the end of three, eighty, ninety, one hundred twenty and one hundred fifty days, are given in table 1.

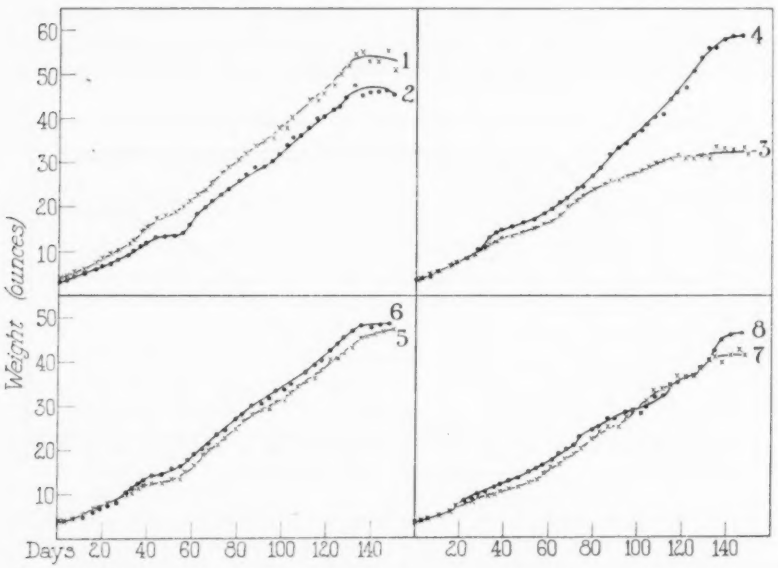

Fig. 2. The average weight of a selected group of chicks grown under amber and corex glass filters, respectively, with and without supplementary irradiation of various types. All chicks were fed the standard Wisconsin all-mash ration. Curves *1* to *6*, chicks housed behind amber glass (Pittsburgh No. 48); curves *7* and *8*, chicks housed behind purple corex glass (Corning G 986 A). The details are given in table 1.

COMMENT. An inspection of the curves of figure 2 and of the data contained in table 1 shows that the average weight of chicks (three days after starting the experiments) in each of the eight compartments was approximately 4 ounces, and that the limits of variation in average weight of chicks were 3.8 (group 6) and 4.5 ounces (group 1). Initial conditions, so far as average weight and the same stock are concerned, were as good as could be expected. The development and growth of chicks, as evidenced by weight, were the least in group 3, in which they were constantly housed

behind the amber filter. Group 1 (curve *1*) consistently evidenced the greatest average weight. A comparison of the data relative to the average weight of chicks housed behind the amber filter and given a supplementary

TABLE 1

The growth and development of chicks in the various groups

GROUP	TYPE OF SELECTIVE IRRADIATION (CHICKS CONSTANTLY HOUSED BEHIND SPECIFIED FILTER)	AVERAGE WEIGHT, OUNCES, AFTER DAYS					ADEQUACY OR INADEQUACY* FOR NORMAL GROWTH AND DEVELOPMENT AS JUDGED BY WEIGHT
		3	80	90	120	150	
1	Amber filter** with irradiation by quartz-mercury lamp† (fifteen minutes daily); heads only exposed	4.5	31	35	47	55	Adequate
2	Amber filter, with irradiation by quartz-mercury lamp (fifteen minutes daily); bodies only exposed	4.1	26	29	42	47	Adequate
3	Amber filter only	4.0	24	26	31	33	Inadequate
4	Amber filter, with irradiation by quartz-mercury lamp through corex‡ filter G 986 A (twenty minutes daily); bodies and heads exposed	4.0	27	33	47	58	Adequate
5	Amber filter, with irradiation by quartz-mercury lamp (five minutes daily); bodies and heads exposed	4.5	25	29	39	47	Adequate
6	Amber filter, with irradiation by quartz-mercury lamp (ten minutes daily); bodies and heads exposed	3.8	27	31	41	49	Adequate
7	Corex filter only	3.9	22	25	36	41	Inadequate
8	Corex filter, with direct sunlight filtered through amber glass (fifteen to twenty minutes daily); bodies and heads exposed	4.1	25	28	37	45	Inadequate

* The judgment regarding adequacy of the radiant energy used to induce normal growth and development is based on the data obtained on a group of chicks which was fed the same Wisconsin ration with 2 per cent by weight of cod liver oil added to the diet and housed constantly behind quartz-containing glass (vitaglass). These data regarding weight are: 3 days, 4 ounces; 80 days, 26 ounces, and 150 days, 51 ounces.

** Pittsburgh amber glass (cathedral) number 48, 3 mm. thick.

† Victor X-Ray Corporation air-cooled quartz-mercury lamp, operated at 90 volts and at a distance of 50 cm. from the chick (intensity of ultraviolet portion of arc under voltage and at distance specified was 575 ergs each second for each square millimeter).

‡ Corex glass, Corning Company G 986 A, unpolished, 7.5 to 8 mm. thick. Intensity of energy from the air-cooled quartz mercury arc, operated at 90 volts and at a distance of 50 cm., transmitted by this filter was 160 ergs each second for each square millimeter.

daily irradiation of fifteen minutes with the quartz-mercury lamp (operated at 90 volts and at a distance of 50 cm.), the heads only being irradiated

(curve *1*) with the data obtained under similar experimental conditions, the bodies only being irradiated (curve *2*), indicates that the irradiation of the heads, with relatively large exposed vascular areas, is more effective (or at least as effective) in promoting growth and development than similar periods of exposure of the bodies only to such irradiation. A superposition of curve *4* of figure 2 (showing the results obtained with supplementary irradiation of twenty minutes daily with an air-cooled quartz-mercury lamp, and the use of purple corex filter) with curve *1* (showing results obtained with a supplementary daily irradiation for periods of fifteen minutes with the air-cooled quartz-mercury lamp, the heads only being irradiated) indicates that the energy from the quartz-mercury lamp transmitted by the corex filter in daily periods of twenty minutes is about as efficacious in promoting growth and development as the total irradiation

TABLE 2

The indexes of refraction (Becke method) of calcified tissues (femurs) of chicks housed behind amber and corex filters, with and without the supplementary periods and types of irradiation given in table 1

GROUP	CHICKS THREE MONTHS OLD		CHICKS FIVE MONTHS OLD	
	Index as first determined (± 0.003)	Index after five days of drying at 60°C. (± 0.003)	Index after five days of drying at 75°C. (± 0.003)	Calcium, per cent
1	1.541	1.557	1.559	19.8
2	1.552	1.558	1.558	20.0
3	1.546	1.549	1.552	23.8
4	1.540	1.562	1.556	23.9
5	1.543	1.550	1.553	23.1
6	1.552	1.559	1.559	20.7
7	1.554	1.559	1.559	21.3
8	1.552	1.559	1.557	22.1

from the bare quartz-mercury arc. Curves *5* and *6* show that supplementary irradiation of the whole bodies of the chicks for periods of five or ten minutes daily with the quartz-mercury arc is not as effective as irradiation for fifteen minutes daily with the same quartz-mercury lamp, the heads only being irradiated. However, development and growth, as judged by weight, may be considered normal in groups 5 and 6. Curve *7* shows that constant housing behind purple corex glass, which transmits a percentage of the ultraviolet energy in the region of 400 to 290 millimicrons but practically no visible solar energy, does not promote normal development and growth. A supplementary exposure of chicks, housed behind corex glass, for fifteen minutes to direct sunlight filtered through the amber glass number 48 (curve *8*), is not sufficient to produce results comparable to those shown by groups 1, 2, 4, 5 and 6. The data in table 1 and the curves

of figure 1 indicate that the greatest growth and development, as evidenced by weight, occurred when the chicks, which were housed constantly behind the amber glass filter, received, for ten to fifteen minutes daily, irradiation with the air-cooled quartz-mercury lamp operated at 90 volts and at 50 cm. from the chicks (intensity of ultraviolet portion of the quartz-mercury arc being about 575 ergs each second for each square millimeter of surface irradiated).

MICROSCOPIC INVESTIGATION OF INDEXES OF REFRACTION OF CALCIFIED TISSUES. The femurs of eight chickens about three months of age, one from each group, were examined by the microscopic method (Becke effect) of determining indexes of refraction (4). The values obtained are given in table 2. The second set of figures obtained from chicks three months old, is given in addition to the first set to show the important influence of thorough drying. In every case the index was raised considerably by drying. The values obtained after five days' drying are to be taken as correct. Although most of the bones gave indexes of 1.559 ± 0.003, those from groups 3 and 5 gave values of 1.550 ± 0.003. The difference is three times the experimental error. The value of the refractive index for bone may be taken as 1.56. The index of refraction may serve as a measure of the degree of calcification of dried tissues, and it is probable that the index is determined by the relative amount of inorganic crystal and organic material in the unit structures. We believe, therefore, that the calcification of the femurs of chicks housed constantly behind the amber glass (group 3) and of those receiving supplementary irradiation with the quartz-mercury lamp for five minutes only each day (group 5) was not as complete as in the other groups in which larger doses of irradiation from the quartz-mercury lamp were given or the chicks were housed constantly behind the ultraviolet transmitting filter, purple corex (Corning 986 A).

SUMMARY AND CONCLUSIONS

The experimental conditions employed, the grade of stock used, and the ration fed in our investigations resulted in data as follows:

1. Constant housing of chicks behind a filter (red-purple corex) which transmits practically only the ultraviolet region (400 to 290 millimicrons, with maximal transmission of 20 per cent at 330 millimicrons) does not permit normalcy in development and growth as evidenced by weight.

2. Constant housing of chicks behind an amber glass filter which transmits only the longer visible wavelengths of sunlight (500 millimicrons to the end of the visible spectrum) definitely retards growth and development.

3. Supplementary irradiation of chicks, constantly housed behind a filter (amber) which transmits only the long visible portion of the spectrum, with an air-cooled quartz-mercury lamp operated at 90 volts and at a distance of 50 cm. (ultraviolet irradiation of energy approximately 575

ergs each second for each square millimeter) for ten to fifteen minutes a day induces normalcy in growth and development.

4. Supplementary daily periods of twenty minutes of irradiation of chicks, constantly housed behind the amber filter, with the energy of a quartz-mercury lamp (operated at 90 volts and at a distance of 50 cm.) transmitted by purple corex glass (160 ergs each second for each square millimeter) induces normal growth as evidenced by weight, but does not permit normalcy of parathyroid glands.

5. Irradiation with the ultraviolet portion (incident ultraviolet energy being 575 ergs each second for each square millimeter) of a quartz-mercury lamp for periods of ten to fifteen minutes a day is capable of producing normal growth and development.

6. Ultraviolet irradiation of the heads only of chicks is apparently as effective as (and possibly more effective than) irradiation of the bodies only in establishing normal development and growth, the chicks being housed behind an amber filter.

7. Microscopic investigations (Becke method) on the calcification of tissues (femurs) in chicks about three and five months old, respectively, housed behind amber and corex filters and given various supplementary amounts of irradiation with the quartz mercury-arc lamp, showed that normal calcification does not occur in chicks receiving only the long wavelengths of sunlight.

8. The data on growth and development, as judged by weight, closely parallel the degree of normalcy evidenced in the development of the parathyroid glands. Hyperplasia of the parathyroid glands is apparent in chicks housed behind either amber glass (transmitting long visible wavelengths) or corex glass (transmitting ultraviolet), and is least evident in chicks that have received irradiation ten or fifteen minutes daily with the air-cooled quartz-mercury lamp.

9. Further evidence is presented, therefore, to support our statement that normal growth and development are dependent on both the ultraviolet and visible portions of solar irradiation, all other factors remaining constant so far as is known.

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FURTHER INVESTIGATIONS ON THE EFFECTS OF RADIANT ENERGY ON THE DEVELOPMENT OF THE PARATHYROID GLANDS OF CHICKS

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During the summer of 1927, we (1), (2) conducted a series of experiments on the effects of selective solar irradiation on the growth of chicks and on the development of parathyroid glands of chicks. In these experiments chicks were placed in compartments that were fitted with windows having a southern exposure and about 10 square feet of amber glass (Pittsburgh no. 48), blue glass (Pittsburgh no. 56), vitaglass, and ordinary glass filters, respectively. Half of the chicks under each filter were fed the Wisconsin basic all-mash ration; the other half were fed this ration plus 2 per cent by weight of cod liver oil. Hyperplastic parathyroid glands developed within the first few weeks in young chicks growing under the amber and the blue glass filters, when they were fed the basic ration only; more nearly normal glands developed in chicks that were provided with 2 per cent by weight of cod liver oil added to the ration. Normal glands developed in chicks growing under quartz-containing glass (vitaglass) which is known to transmit a large percentage of the shorter wavelengths of sunlight. Also in chicks under ordinary window glass, normal parathyroid glands developed when the chicks were fed the basic ration plus the cod liver oil. Normal ratios of blood calcium and phosphorus were maintained even in the chicks grown under the blue and the amber filters. Signs of weakness in legs or deficiency in bone were not manifest until late in the summer or after ten to twelve weeks of experimental confinement. We concluded from these experiments that both the shorter and the longer wavelengths of sunlight are essential to the development and the normal growth of parathyroid glands.

The results of these initial experiments prompted us to carry on a second series of investigations in which we attempted to compensate for the absence of the shorter wavelengths of solar radiation, which are not transmitted by amber glass filters (Pittsburgh no. 48), through a daily exposure

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of the chicks (housed behind such filters) to the radiant energy of an air-cooled quartz-mercury-arc lamp.

EXPERIMENTAL PROCEDURES. Eight groups of chicks, fifteen in each respectively, were isolated in compartments, six of which were screened from the full complement of direct sunlight by the use of amber filters which transmitted less than 1 per cent in the near ultraviolet portions and about 80 per cent in the orange and red portions of the spectrum. Two of the compartments were fitted with windows carrying the red-purple corex glass (8 square feet), which transmits radiant energy from about 270 to 400 millimicrons, thus deleting practically the whole of the visible spectrum (slight transmission of red) but transmitting an appreciable amount of the ultraviolet region of sunlight.

The chicks were confined within these compartments throughout the five months of the experiment, except for certain daily periods of supplementary irradiation by the quartz-mercury lamp. All chicks were thus subjected to such radiant energy only as was transmitted by the respective filters, supplemented by irradiation with the air-cooled quartz-mercury arc. This supplementary exposure to irradiation by the quartz-mercury lamp (operated at 90 volts and at a distance of 50 cm.) varied as follows: The heads of all chicks in group 1, housed behind the amber filter, were irradiated for fifteen minutes each day, except Sunday; while the bodies only of all chicks in group 2, also screened by the amber glass, were irradiated for the same period and at the same distance. This variation in the site of irradiation was made in an attempt to determine whether the head, with its highly vascular combs and wattles, was any more receptive to the shorter wavelengths of the lamp's spectrum than the body generally. The chicks in group 3, housed behind the amber filter, served as the control group which were restricted throughout the course of these investigations to the energy transmitted by the amber filter only. All chicks in groups 4, 5 and 6, also screened by amber filters, were irradiated by the mercury arc for daily periods which varied as follows: Chicks in group 4 were irradiated for twenty minutes daily through the corex filter which transmits radiant energy from the quartz-mercury lamp lying between approximately 270 and 400 millimicrons. The far ultraviolet portion and the whole of the visible portion of the spectrum of the quartz-mercury lamp were thus screened out. All chicks in group 5 were subjected to the radiant energy contained in the entire spectrum of the quartz lamp for daily periods of five minutes; the chicks in group 6 were similarly irradiated for ten minutes daily. The chicks in group 7 were confined continuously behind the corex filter; those in group 8, also continuously confined behind the corex filter, received a supplementary irradiation of summer sunlight through the amber glass for twenty minutes each day.

All chicks were fed the Wisconsin all-mash ration without the addition

of 2 per cent cod liver oil. Occasional determinations of blood calcium and phosphorus were made. A chick from each group was killed at the end of two weeks, one month, two months and three months, respectively, and all remaining chicks were killed at the end of the experiment. Portions of the thymus gland and all of the thyroid and parathyroid glands were carefully removed, fixed in Bouin's solution, sectioned and stained in hematoxylin and eosin.

EXPERIMENTAL OBSERVATIONS. The parathyroid tissue of chicks, living outdoors, under essentially normal conditions, is arranged in four glands, two on each side, usually at the posterior pole of the thyroid gland. Each gland comprises groups of cells arranged in cords or columns, separated by a delicate fibrous stroma. The principal cells are ovoid or spheroid and contain large oval nuclei with an irregular chromatin network. A fibrous capsule, four to six cells thick, encases each gland and forms a mesenterial attachment by means of which the gland is suspended. Parathyroid bodies are epithelial in origin, arising from pharyngeal pouches. Occasionally one encounters in a normal gland remnants of a persistent duct. These structures, lined by cuboidal or columnar epithelium, are interpreted as embryonic rests and bear no relation to the conditions of this experiment.

A differentiation in the organization of the parathyroid bodies in chicks of these various groups was visible as early as two weeks after the commencement of the experiments. The parathyroid glands were essentially normal in the chicks of groups 1 and 2. Some hyperplasia was found in chicks of group 1, and in both groups 1 and 2 a small increase in the interstitial fibrous connective tissue had occurred. Glands from chicks of group 3 (the control group without supplementary irradiation with quartz-mercury lamp) were already twice the size of those in the chicks grown in direct sunlight out of doors. In the chicks in group 4, which received a supplementary irradiation with the quartz-mercury lamp through the corex filter marked changes were manifest. Associated with marked hyperplasia there were regressive changes with cystic formation and fibrous invasion. The absence of the shorter wavelengths of the ultraviolet region caused early and marked changes in the development of the parathyroid glands.

The parathyroid glands of the chicks in group 5 and 6 (which were given supplementary irradiation for five and ten minutes daily with the quartz-mercury lamp) were in a more nearly normal state. So far as organization of the gland and its framework of connective tissue are concerned, it was impossible to distinguish these glands from those of chicks grown out of doors under natural conditions. The glands in the chicks of group 7, housed behind the corex filter, were twice the size of the glands in chicks of groups 5 and 6 to which supplementary irradiation with the quartz-mercury lamp of five and ten minutes each day, respectively, were given. There was no essential difference between the glands of chicks in groups 7

and 8 (corex filter) although the degree of hyperplasia in the latter group was less pronounced and it would appear that the brief exposure of twenty minutes daily to the longer wavelengths of sunlight was not without effect.

At the end of two weeks of experimental observations, therefore, certain structural modifications had developed within the parathyroid glands of the eight groups which suggested rather definite reactions to the character of the radiant energy provided. Taking as standard the cytology of the parathyroid glands of a normal healthy chick grown out of doors, it was evident that the experimental group 6 (constantly housed behind the amber filter, with a supplementary ten-minute daily irradiation by the quartz-mercury-arc lamp) approximated normalcy or was essentially identical with the normal. The parathyroid glands were of the expected size and there were no gross or microscopic abnormalities. In the absence of the shorter wavelengths of solar radiant energy (group 3) or the absence of the longer wavelengths (group 7) marked hyperplasia had taken place without cystic degeneration or fibrous invasion. However, the twenty-minute daily supplementary irradiation with the longer wavelengths of sunlight given to chicks in group 8 apparently partially compensated for these losses, as was evidenced by the more nearly normal state of the gland. When supplementary irradiation by the entire spectrum of the lamp was given to the chicks growing under the longer wavelengths of solar irradiation (groups 5 and 6) normal parathyroid glands developed that were comparable in every respect to those of the outdoor chicks. At this early stage of experimentation the five-minute daily period of irradiation was as effective as the ten-minute period. Furthermore, the irradiation of the heads only (group 1) or the bodies only (group 2) was without significant differential effect on the development of the normal structure of these glands. Some hyperplasia had taken place, but otherwise a normal gland had developed. Accordingly, it seemed evident that the energies contained in the ultraviolet region as well as that present in the longer visible end of the spectrum were essential to normal growth of the parathyroid glands.

The reaction of the parathyroid glands in chicks growing under the amber filter but receiving daily supplementary irradiations of the lamp through the corex filter (group 4) was as might be expected. The extensive hyperplasia was not unlike that present in the glands of chicks in group 7 (continuously housed behind the corex filter) but the accompanying degenerative, regressive changes were further evidences of functional disturbance. Since the experimental conditions surrounding the chicks in groups 1, 2, 4, 5 and 6 were alike in that supplementary irradiation by a quartz-mercury lamp was added to the solar irradiation received through the amber filters, the pathologic condition developing in glands of chicks in group 4 may have been due either to the absence of the shorter ultraviolet region which was screened out by the corex filter (which transmits from 270 to 400

millimicrons) or to the fact that this filter does not transmit more than 20 per cent even at its maximal point of transmission (330 millimicrons). Longer wavelengths of solar irradiation, supplemented with irradiation by a quartz-mercury arc for periods as short as five minutes a day, were adequate to maintain normal parathyroid glands in chicks kept on a ration in which there was a deficiency of vitamine D.

After one month of experimentation, all chicks in all compartments were in an apparently healthy condition. There was no evidence of weakness of the legs even in the chicks of the control group, which were continuously confined behind the amber filter. The glands of the chicks that were killed from groups 5 and 6 which were given the daily supplementary irradiation by the quartz-mercury lamp for five and ten minutes, respectively, continued to be normal, at least so far as gross and microscopic data are concerned. The hyperplasia of the parathyroid glands of chicks taken from the control group 3 was even more marked, so that these glands were grossly four to five times the size of those in a chick of the same age living out of doors in full summer sunlight. With the hyperplasia, there was marked fibrous invasion and leukocytic infiltration, whereas the usual columnar or unit arrangement of the cells was lost to a large extent. In one of the enlarged glands there was a large cyst (not a persistent duct) lined with cells which were comparable in every respect to the principal gland cells. Thus, a cystic type of regression, such as we encountered in our earlier series under identical conditions, was in evidence. The parathyroid glands of chicks from groups 1 and 2, in which the solar energy transmitted by the amber screen was supplemented by irradiation of the head and body, respectively, with the mercury arc, were essentially normal and compared favorably with the outdoor controls. In the chicks in group 4 the same abnormality as was present in the glands of these chicks at the end of two weeks was present also after four weeks. One gland, however, was normal, but the others presented marked hyperplasia and extensive cystic degeneration with polymorphonuclear leukocytic infiltration and excessive fibrous invasion.

In the glands of chicks grown under the corex filter (groups 7 and 8) the pathologic change was even greater than at the end of the two weeks period. We failed to detect, however, any pronounced difference in the glands of chicks given the supplementary twenty-minute daily solar irradiation through the amber filter. Most characteristic, perhaps, of glands of chicks from both groups 7 and 8 was the excessive hyperplasia of the principal cells. Instead of the usual two pairs of glands at the posterior pole of the thyroid body, these chicks had a multilocular gland, formed of several functional parts held together by strands of connective tissue fibers. Cell columns retained their identity and were separated from each other by an extensive fibrous network. Cysts abounded but they were far less prominent than in the glands of chicks from group 4.

After one month of experimental control, the parathyroid glands of these eight groups of chicks may be classified rather definitely in two groups on the basis of their structural organization. In chicks grown under the amber filter and given supplementary irradiation with the entire spectrum of the quartz-mercury arc, ranging from five minutes to fifteen minutes daily, restricted either to the head or the body, parathyroid glands developed essentially comparable to those of chicks grown outdoors in full midsummer sunlight. In chicks given the supplementary mercury-arc irradiation through the corex filter (group 4), marked hyperplasia, fibrous invasion and cystic degeneration developed in the parathyroid glands. These glands resembled in essential detail (except for the degree of cystic degeneration) the glands of chicks housed under the corex filter continuously (group 7) as well as of those grown under the corex supplemented by the daily solar irradiation through the amber filter (group 8).

After three months, the parathyroid glands of chicks housed in these various compartments showed structural differences, correlated no doubt largely with environmental factors imposed by the experiment. It is well to bear in mind, however, that diseased parathyroid glands may develop under conditions entirely foreign to deficiency in diet. The glands which were more nearly comparable to those of a chick growing in out-of-door sunlight, which we may call normal, were those in the chicks in group 6 which were given the ten minutes' supplementary irradiation each day with the quartz-mercury arc. There was essentially no hyperplasia, no cystic formation, and only slight fibrous development. Five-minute daily irradiation with the quartz-mercury lamp was hardly sufficient to maintain normalcy of the parathyroid glands. Chicks from group 3 (amber filter only) had hyperplastic parathyroid glands, with marked fibrosis but without cystic degeneration.

The most marked pathologic change of the parathyroid glands at the end of the period of three months was encountered in chicks in groups 1 and 2 which are irradiated (head and body respectively) with the quartz-mercury lamp for fifteen minutes daily. Hitherto these glands had been essentially normal, but at this stage and for the remainder of the experiment certain of the glands in both groups showed pathologic change. In each chick examined, the anterior gland of each pair of parathyroid glands was normal in that a normal capsule enclosed normal cords of cells arranged as in any parathyroid gland that is functioning. The posterior gland, however, was greatly enlarged and contained only a central core of what could be considered functioning tissue. Surrounding this central zone was an area in which numerous multilocular cysts were imbedded in an extensive stroma of connective tissue and blood cells. Throughout the stroma of this adenoma-like structure were scattered parathyroid cells, some of which were still grouped into typical nests. Polymorphonuclear leukocytes were

abundant. The wide distribution of the principal cells throughout this area led us to conclude that this form of regression followed marked hyperplasia. On the basis of the types of cysts we encountered, we believe that the condition could well be designated as multiple cystic adenomatous dilatation. This condition appeared in chicks of both groups 1 and 2, and thus could not be associated with the site of irradiation, nor could we definitely assign the condition to the character of the irradiation. We feel that it is not due to insufficient exposure, for it did not appear in groups 5 and 6. In the chicks that served as controls under the amber filter (group 4), something of the same cystic dilatation appeared, although to a far less extent; thus we feel that it could not be associated with excessive irradiation with energy from the region of the short wavelength of the quartz-mercury arc. The causes are problematic and may not be associated with irradiation per se. The lesion present in group 4 appeared to be more of the character of hyperplasia and fibrous development than of cystic dilatation. The chicks in groups 7 and 8 continued in good physical condition, and yet their parathyroid glands were abnormally large. Tumor-like formations, such as we encountered in glands of chicks from groups 1 and 2 and to a limited extent in those from group 4, were not found in glands from chicks of groups 7 and 8. It is possible that these aberrant growths may be correlated slightly with the effects of the irradiation and possibly are due to other extraneous factors.

COMMENT. These experimental observations further substantiate the conclusions reached in our previously reported investigations to the effect that hyperplasia of the parathyroid glands develops whenever a chick is denied vitamine D either in radiant energy or in diet. Since metabolism of calcium is correlated in some way with the function of the parathyroid glands it would appear that nature attempts to compensate for the absence of the accessory dietary factor by increasing the total functional activity of the gland. Hyperplasia was the most marked in group 3 (the control group under the amber filter) and, toward the end of the experiment, when weakness of the legs was pronounced and other signs of deficiency in metabolism of calcium were manifest, extremely large parathyroid glands were present. Frequently these glands were half the size of the thyroid glands. Hyperplasia developed early and was recognized during the first two weeks of experimental control. Rapid proliferation of cells persisted and varying degrees of regression, cystic degeneration and fibrous invasion continued.

In our previous experiments (1), (2) hyperplasia of these glands was present in proportion to the degree of availability of the necessary dietary factor (vitamine D). Varying degrees of hyperplasia ensued, depending on the extent to which the vitamine D was a factor in the source of radiant energy provided. The addition of 2 per cent by weight of cod liver oil to

the standard diet, although not as effective as the entire spectrum of midsummer sunlight as transmitted by vitaglass, nevertheless maintained in chicks, confined behind amber glass, more nearly normal parathyroid glands. In our present experiments a basic diet, without the cod liver oil increment, was given to all chicks and the attempt was made to compensate for the absence of the accessory factor in the diet by varying amounts of supplementary irradiation with a quartz-mercury-arc lamp. In this way we hoped to determine just what was the optimal exposure necessary to maintain morphologically normal parathyroid glands in chicks in which otherwise glands would develop with varying degrees of pathologic change. We also wished to determine whether the head of the chick, with its highly vascular comb and wattles, was any the more receptive to radiant energy, as revealed in the reactions of the parathyroid glands to such energy, than the body and legs, with their feathers and cornified protection.

On the basis of our observations extending over a period of five months, during which 125 chicks were killed and examined for data on the parathyroid glands, certain deductions may be made. First, it is clear that hyperplasia, and not hypertrophy, ensues when chicks do not receive both the longer visible wavelengths of sunlight and the ultraviolet portion of either sunlight or of the quartz-mercury lamp. In chicks in group 3 (the control flock), in chicks in group 4 (those irradiated through the corex), and in chicks in groups 7 and 8 (those continuously confined behind corex filters), hyperplasia of the parathyroid glands developed at a very early stage. This hyperplasia varied in extent dependent on the presence or absence, as well as on the quantity, of the longer or shorter wavelengths of light. In chicks in group 8, which were irradiated with ultraviolet energy (corex) in the region of 290 to 400 millimicrons supplemented with irradiation twenty minutes daily with noonday sunlight of the longer visible character, glands developed that were more nearly normal than those of chicks in group 7 (housed behind corex). Likewise in chicks in group 4 continuously exposed to the energy of the longer wavelengths of sunlight through the amber filter and irradiated with the ultraviolet portion of the mercury lamp transmitted by corex for brief daily periods, glands developed more nearly normal than in those in either group 3 (the controls behind amber glass) or those in group 7. In all four groups, hyperplasia of the parathyroid glands was followed by various forms of degeneration, such as cystic formation, fibrous invasion and leukocytic infiltration. Nothing was seen that could be considered as in any way constant for the type of regression which ensued within these hyperplastic glands.

The energy transmitted by solar irradiation through an amber filter supplemented by daily ten-minute periods of mercury-arc irradiation appeared to compensate satisfactorily for the absence of vitamine D either in sunlight or in diet, at least as far as the morphology of the parathyroid

glands is concerned. Chicks, which were given this supplementary irradiation, maintained throughout the duration of the experiment a gland that was essentially comparable to those grown out of doors. On the other hand, five-minute daily periods of irradiation were ineffective in maintaining glands as nearly normal, since all chicks in group 5, especially later in the experiment, gave evidence of some degree of hyperplasia.

The lesions that developed in the parathyroid glands of chicks in groups 1 and 2 cannot be explained readily. The glands of chicks that were killed during the first month of the experiment were normal in essential cytologic details and it appeared to us that the fifteen-minute daily irradiations of the head of the body were sufficient to maintain normal structure. There appeared to be no difference in either the growth and development of the two groups or of the finer cytology of the parathyroid glands and thus irradiation of the bodies and legs was as effective as that of the heads only. However, after two months of experimental control, we did not find normal glands in the chicks of either group 1 or group 2. We are unable to say whether there were other factors to confuse our interpretation. These chicks were maintained on the same food and water ration as all the others, and their general health continued good. So far as we know, the character of the irradiation was the only differential factor in the environment of these chicks. And yet marked hyperplasia and an adenomatous type of cystic dilatation were encountered that were not encountered in the chicks of groups 5 and 6. It seems strange that the same type of lesion should arise in the chicks of these two groups which were given comparable periods of irradiation.

SUMMARY AND CONCLUSIONS

1. Normal parathyroid glands developed only in the presence of both the visible and ultraviolet portions of radiant energy.

2. Normal parathyroid glands developed in chicks constantly housed and grown behind an amber filter and given daily supplementary irradiation of ten or fifteen minutes with an air-cooled quartz-mercury lamp. Five-minute periods apparently did not compensate fully for the absence of the vitamine D factor in the diet or induce normal development under sunlight filtered through amber glass.

3. The ultraviolet portion of sunlight transmitted by corex glass (limits of transmission from 270 to 400 millimicrons with a maximal transmission of 20 per cent at 330 millimicrons) was not sufficient to compensate for the absence of vitamine D in the diet or the ultraviolet content of unfiltered sunlight. Normalcy of parathyroid glands was not present.

4. Our experiments showed that if, during the first two months, chicks were given daily irradiation for ten to fifteen minutes with the air-cooled mercury arc (intensity of 575 ergs each second for each square millimeter),

applied either to the heads or the bodies of chicks constantly housed behind a filter transmitting only the longer visible portion of the spectrum, essentially normal parathyroid glands developed and were maintained. During the third to the fifth months, however, pathologic conditions of various types and extent developed.

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ACTION OF THE VAGUS NERVES ON THE CHRONAXIE OF THE AURICLES AND THE VENTRICLES OF THE TURTLE HEART; RELATIONSHIP TO CHROMOTROPIC CHANGES

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There can be no doubt that faradic stimulation of the vagi produces definite reduction in the chronaxie of the cardiac tissue as tested by the method of extra systoles induced by the galvanic current (H. Fredericq, (4), (5), (10), (13); M. Lapicque and C. Veil, (18); Field and Bruecke, (1); Rylant, (20); H. Fredericq and Brouha, (14)).

The chronaximetric method has given us information which cannot be obtained when the bathmotropic changes are determined by the method of threshold stimuli (9). We are dealing with a special example of a general phenomenon in which there is a modification of the celerity of excitability (chronaxie) of tissues, a modification induced by the so-called "vegetative" nerves; this is shown for example in the bathmotropic changes which have been reported upon stimulation of the cardiac accelerators (6), of nerves modifying the gastric contractions (2), (3), and nerves of the splenic plexus (11), (12), all of which modify in one direction or another, the chronaxie of the contractile tissues which they innervate.

Previous studies of the effects of vagus upon cardiac chronaxie have been made especially upon the ventricle. The usual methods employed in the study of the heart have failed to demonstrate any inhibitory vagus fibers in the ventricle of the turtle, and they appear to be present only to a very minor extent in the ventricles of mammals. The question, therefore, arises, viz.: Does faradization of the vagus cause the change in chronaxie by virtue of a direct bathmotropic action of vagus fibers on the cardiac muscle, or is the change in chronaxie the indirect consequence of the chronotropic change, i.e., the decrease in rate?[3] The vagus innervation of the auricles and the auriculoventricular junction is established but the

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[3] Conversely, one might ask whether it is not the change in chronaxie which causes the slow rhythm. In this case one might perhaps, by calling upon the hypothesis of a heterochronism supervening between two links of the automatic cardiac system, disclose the explanation of the inhibition itself.

reports of the effects of vagus stimulation on the chronaxie of these tissues, which include studies by M. Lapicque and C. Veil (18) on the auriculo-ventricular junction of the frog, by Rylant (20) on the isolated auricles of the cat, and by Fredericq and Brouha (14) on the auricles of the eel, have not answered the question which we have raised. The experiments reported in this paper were done to obtain a direct answer to this question. Experiments bearing indirectly on this question are found in a study of the action of certain poisons which alter the cardiac rhythm. The chronaxie of the ventricle has been determined by Henri Fredericq (7), (8), who concludes that there is no necessary parallelism between the changes in rhythm and chronaxie. Furthermore, Field and Bruecke (1) as well as Henri Fredericq (8) have observed independently and simultaneously that during the faradization of the vagus of the frog and of the turtle, the decrease in chronaxie of the ventricle is evident even if the rhythm is restored to the normal rate by induction shocks of appropriate frequency applied to the sinus venosus.[4] Except for these reports on changes in chronaxie, Gaskell's (15) original statement that there is no indication of the slightest direct inhibitory effect of the vagus upon the ventricular muscle of the turtle has been confirmed by every one investigating this question (16). Garrey has shown that the chronotropic slowing of the ventricles is not due to a direct chronotropic vagus action on the ventricles themselves. The sinus and auricles suffer true inhibition with the well known reduction in characteristic properties outlined by Englemann and by Gaskell; the ventricles, on the other hand, are *arrested* merely as a result of failure of these basal structures to function when the vagus is stimulated. The ventricular muscle in this arrested condition will respond to a *reduced* threshold induction shock and after the period of rest contracts more strongly than when beating at the normal rate—conditions which are exactly opposite to those associated with inhibition. It was desirable, therefore, to verify the effects which vagus stimulation may produce on the chronaxie of the turtle's ventricle since they constitute the only known known action of the vagus on this structure.

In order to test the relation of the vagus action on chronaxie, independently of changes in rate of rhythm, we have availed ourselves of the fact, first reported by Meyer (19), that in Testudo sp. the rate is controlled by the right vagus, that there is no chronotropic action of the left vagus. This difference in the action of the two vagi has been studied in detail by Garrey (17), who found on various species of the genus Chrysemys and on other genera of turtles, that there is a predominant effect of each vagus on

[4] At the XIIIth International Congress of Physiologists (Boston, August 1929), a question was raised in discussion concerning the effects of vagus stimulation upon the chronaxie of the heart, whether in reality they were due to a direct effect on inhibitory nerve on the muscle or only indirectly a result of effects on rhythm.

its homolateral side of the heart (sinus and auricles). Since the pace maker of the heart lies on the right side, the left vagus often fails to have any chronotropic effect, although the contractions of the left auricle are markedly weakened, in fact with strong stimulation of the vagus this

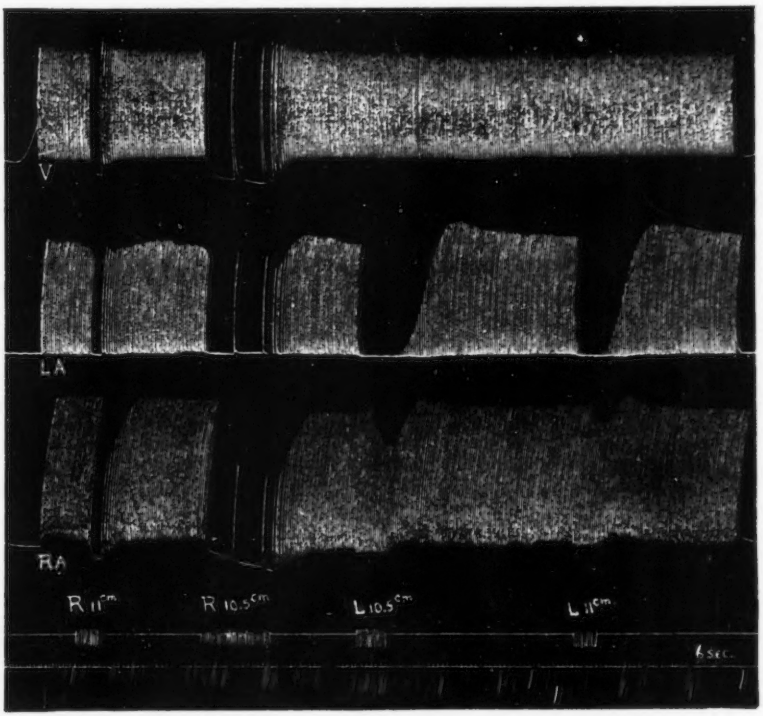

Fig. 1. Tracings of the ventricle *V*, right auricle *RA*, and left auricle *LA* of a turtle's heart. Stimulations of the right vagus at *R* 11 cm. and *R* 10.5 cm. show chronotropic effects, with greater inotropic decrease in the contractions of the right auricle. Stimulations of the left vagus at *L* 10.5 cm. and *L* 11 cm. are without chronotropic effects but with auricular inotropic decrease, especially marked in the contractions of the left auricle. Neither vagus caused any decrease in the height of ventricular contractions. Stronger ventricular contractions are evident with slow rates following right vagus stimulation.

auricle may fail to show any contraction whatever; to a lesser extent the contractions of the right auricle are weakened by the left vagus, although the rate of contraction may be unaffected and the ventricle continue to beat vigorously. By judicious adjustment of the strength of left vagus

faradization the only effect noticeable is the weakening of auricular contractions, more noticeable on the left side than on the right side. These effects are shown in figure 1.

TECHNIQUE. The work was done upon preparations of Chrysemys picta. The vagi and heart were carefully exposed. The chronaxie of the beating auricles and ventricles was determined during the period of normal diastole, then in diastole during stimulation of the vagus nerves. The measure of the chronaxie was made by the usual method of stimulating by closing a constant current and interrupting the circuit after the desired time interval. The excitation circuit consisted of: 1, dry cells; 2, a potential divider of Lapicque; 3, a Lapicque chronaximeter; 4, a resistance of 7000 ohms introduced in series, and 5, stimulating electrodes. The active electrode (negative pole) of the stimulating electrodes was a fine silver wire hooked into the myocardium of either the auricle or ventricle. The indifferent electrode (positive pole) was a bar of silver introduced into the visceral cavity of the turtle. To stimulate the pneumogastric nerve, it was placed on the kaolin electrodes of Lapicque which have the advantage that the nerve is not in contact with metal and the conditions of moisture remain constant. These electrodes are introduced into the circuit of the secondary coil of an inductorium activated by an independent circuit.

The measure of chronaxie is made with the usual precautions: 1, measurement of the rheobasic voltage; 2, measurement of chronaxie with double the rheobasic voltage, and 3, a check measurement of the rheobase. The rheobase before and after the determination of the chronaxie, i.e., 1 and 3, must be identical or the determination is rejected. When the rheobase determinations check the chronaxie is considered satisfactory. All recorded values, those of rheobasic voltage as well as those of chronaxie time, are measured by proceeding from the liminal to the supra-liminal values. The results of the experiments are given in table 1.

ANALYSIS OF RESULTS. Faradization of either vagus consistently reduces the chronaxie of the auricles of the turtle. In contrast to this effect the variations in the rheobase are not consistent. The reduction of the chronaxie takes place even when vagus stimulation fails to cause any change in the rate of the cardiac rhythm, although there is a concomitant decrease in the force of the auricular contractions. Concerning the effects of vagus stimulation upon the chronaxie of the ventricles, the number of observations does not permit us to speak with finality; there was no change in the rhythm of the ventricle when the vagus was stimulated and the force of the contractions was not decreased, nevertheless, the changes in chronaxie due to vagus stimulation were in harmony with those previously reported by Henri Fredericq and others, and therefore must be considered as significant although the explanation of the effect must remain entirely conjectural. In the absence of other evidence of vagus action one may

TABLE 1

DIVISION OF HEART EXAMINED	MEASUREMENTS OF:	REST	CONDITION OF VAGUS NERVES					
			Faradization to left vagus	Faradization to right vagus	Rest	Faradization to left vagus	Rest	Faradization to left vagus
Ventricle measurements were not made until the chronaxie was stabilized	Rate of ventricle per minute	36	36	—	0*	0*	—	—
	Rheobase (volts)	1.8	1.8	—	1.45	1.45		
	Chronaxie, σ = 0.001 second	3	2	—	3	3 ā 4		
Left auricle, vagus cut	Rate per minute	36	36†					
	Rheobase (volts)	2.8	2.2					
	Chronaxie, σ = 0.001 second	5	4					
Right auricle, vagus cut	Rate per minute	36–40	—	0	41 ā 42	41 ā 42‡	41–42	41 ā 42‡
	Rheobase (volts)	1.1	—	2.8	1.8, 1.1	1.1	1.1, 1.1	1.1
	Chronaxie, σ = 0.001 second	3	—	1.5	4.5, 4	2	3.5, 3	1.5
Right auricle, vagus cut	Rate per minute	40§	40	—	40	40§	40	
	Rheobase (volts)	2	1	—	1.5	0.6	0.8	
	Chronaxie, σ = 0.001 second	6	2.5	—	8.5	1.5	9	
Right auricle, vagus cut	Rate per minute	38–40	38–40**	—	38–40	38–40**	38–40	38–40**
	Rheobase (volts)	1.2	1	—	1	0.8	1, 0.92	0.75
	Chronaxie, σ = 0.001 second	3	1.5	—	3	2	2.5, 2.5	2

* Ventricular rest produced by crushing of the auriculo-ventricular junction, with eventual interruption of the vagus path at this level.

† No effect of the left vagus on the rhythm of the left auricle. Decrease in height of contraction.

‡ No effect of left vagus on the frequency or height of contraction of right auricle.

§ Faradization of the left vagus decreased the height of the contractions of the right auricle without modifying its rhythm.

** Faradization of the left vagus lessens the force of the contractions of the right auricle without modifying its rhythm.

consider the possibility that the changes in chronaxie are the result of vagus action on a particular property of the ventricle, viz., the celerity of excitability (the chronaxie), without giving evidence of those other changes which we are accustomed to associate with vagus action, i.e., that changes in chronaxie may be obtained independently of changes in other properties of the muscle, and it is quite certain that the changes in heart chronaxie produced by stimulation of the vagi are not the consequence of the chronotropic action of these nerves. We are thus faced with a new problem of explaining the changes in chronaxie due to vagus stimulation. Is it possible that these changes are mediated by nerve fibers in the vagus which are not associated with inhibition as we ordinarily consider it?

RÉSUMÉ

Faradization of the right vagus nerve of the turtle stops the contractions of the heart and, at the same time, diminishes the value of the chronaxie of the right auricle (and probably of the entire myocardium).

The chronaxie of both auricles is likewise diminished during stimulation of the left vagus without any change in the rhythm, although the force of contractions may be appreciably diminished. Ventricular chronaxie is also diminished (confirming previous work), although stimulation of the vagus (left) produces no change in the rhythm or decrease in the force of contractions. Consequently, the changes in chronaxie are due to a direct action of the vagus and are not a result of indirect chronotropic changes.[5]

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[5] We wish to express our indebtedness to the Director and other officers of the Marine Biological Laboratory of Woods Hole, who placed the facilities of this splendid institution at our disposal during the summer of 1929.

EFFECTS OF DIET POOR IN INORGANIC SALTS ON CERTAIN ORGANS AND BLOOD OF YOUNG RATS[1]

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In 1927 Winters, Smith and Mendel showed that, when young rats weighing 40 grams were given an experimental ration poor in mineral salts but otherwise complete, the animals ceased to grow though the body weight was maintained fairly constant throughout the experimental period of 40 days. Along with this failure of normal increase in body weight there was demonstrated a persistent increment in length of the long bones with consequent distortion of the body weight to body length ratio. The weight of the dried bones of the experimental group was also greater than that of normal control animals of the same body weight. Furthermore, there was a uniform and striking enlargement of the kidneys of the rats stunted by the experimental procedure employed. The present report deals with an analysis of the character of the changes observed in bones and kidneys together with new observations on the spleen and blood.

EXPERIMENTAL PART. *Care and selection of animals.* Female rats from the breeding colony of this laboratory were taken at weaning, when the weight was 40 to 50 grams and distributed to the various experimental groups. All animals were kept in individual all-metal cages so constructed that coprophagy was prevented. The routine care of the cages, already described (see Smith, Cowgill and Croll, 1925) was followed. Distilled water and the experimental ration were available ad libitum to all rats except the "low calorie" group whose food was given in weighed amounts. All animals were weighed daily.

Diets. The following four experimental rations were used:

DIET 1		DIET 2		DIET 3		DIET 4	
Food material	Concentration	Food material	Concentration	Food material	Concentration	Food material	Concentration
	per cent		*per cent*		*per cent*		*per cent*
Casein	18	Casein	18	Casein	35	Casein	45
Starch	51	Starch	55	Starch	27	Starch	13
Lard	27	Lard	27	Lard	30	Lard	32
Salts	4			Salts	8	Salts	10

[1] The data reported in this paper have been taken from the thesis submitted by Robert V. Schultz in partial fulfillment for the degree of Doctor of Medicine, Yale University, School of Medicine, 1929.

The salt mixture was that described by Osborne and Mendel (1917). All rats received 75 mgm. yeast concentrate[2] which had been tested in this laboratory for its vitamin-B (complex) potency; in addition, 5 drops of cod liver oil were fed, both adjuvants being given apart from the rest of the food. To eliminate the inorganic salts as far as possible from the "low salt" diet, a lot of casein[3] poor in fat was washed in weak acid (pH 4.7) for 4-hour intervals three times daily and in cold running water over night, for 5 successive days. It was then washed with alcohol, centrifuged and dried in the oven at slightly below 100°C. The resulting product contained 14.6 per cent nitrogen, 1.2 per cent moisture and 0.9 per cent ash.

METHODS. The method of measuring the body and tail length was the same as that employed in the previous study (Winters, Smith and Mendel, 1927). The spleen was always removed as quickly as possible after death of the animal to eliminate, as far as possible, post-mortem change in size (see Barcroft and Stevens, 1927). Organs and bones were dried to constant weight at 105°C., and ashed to constant weight at 450°C., the dried bones being extracted with alcohol and ether before ashing. Blood for the red cell count was obtained from the tail in most cases. Hemoglobin was determined by the method of Cohen and Smith (1919) upon blood drawn from the heart at autopsy. The pooled heart blood from several rats was used for determining plasma total nitrogen and red cell volume. The diameters of the erythrocytes were measured with an eyepiece micrometer.

Experimental groups of animals. The plan of the study was to compare, on the basis of measurements and of composition of the various organs, the group given the low salt ration with other groups of animals of the same age or body weight but with different nutritive history during the experimental period (40 days). These groups served as controls.

The "low salt" group, consisting of 26 rats weighing from 41 to 49 grams, was given diet 2 without added salts at weaning and in all but one case the body weight was maintained at 45 ± 5 grams throughout the experimental period (40 days). Occasionally the weight of a rat in this group dropped below the 40-gram limit but administration of the control diet containing salts always succeeded in restoring the weight level above 40 grams in a day or two.

The "physiological" control group comprising 20 animals was killed and autopsied at weaning when they weighed 40 to 45 grams. These were normal rats of the same *body weight* as that maintained in the "low salt" group.

The "chronological" control group consisted of 12 rats which had been given diet 1 at weaning and which grew normally on this ration for 40 days, the same length of time that the "low salt" group was kept at constant body

[2] Yeast Vitamine (Harris), lot 1030.

[3] No. 453, Casein Manufacturing Co., New York.

weight. There were thus available normal animals of the same *age* as that of the experimental group.

The "low calorie" control group included 10 rats maintained at the weaning weight—40 to 45 grams—for the 40-day experimental period by limiting the quantity of an adequate ration fed. This group was given diets 3 and 4 which are so adjusted that, in spite of the decrease in total calories provided when 1 to 2.5 grams of the food are fed, the absolute quantity of both the indispensable protein and salts is adequate for normal growth of a rat weighing 40 to 45 grams. At the begining of the experimental period this group required about 2.5 grams of food per day and diet 3 was used; later the energy requirement for maintenance of body weight decreased to approximately that furnished by 1 gram of food and then diet 4 was fed. Both rations yield about 5 Calories per gram. This group served as a control on the effects of stunting *per se*, apart from any qualitative deficiency in the ration.

TABLE 1

Body weight, body length and tail length of the various groups

GROUP	MEAN BODY WEIGHT	COEF. VARIATION	MEAN BODY LENGTH	COEF. VARIATION	MEAN TAIL LENGTH	COEF. VARIATION
	grams	*per cent*	*mm.*	*per cent*	*mm.*	*per cent*
"Low Salt"	44±2.5*	5.7	118±6.5	5.5	99±5.3	5.4
"Physiol." Control	41±1.9	4.5	112±3.4	3.0	74±3.8	5.1
"Chronol." Control	143±3.9	2.7	174±3.3	1.9	139±7.0	5.0
"Low Calorie"	47±0.9	1.9	124±2.1	1.7	111±2.8	2.5

* Standard deviation.

The "low salt" splenectomized group comprised 10 rats, splenectomized at weaning and maintained thereafter on diet 2 during the 40-day experimental period.

DISCUSSION OF RESULTS. *Body weight, body length and tail length.* As demonstrated in the previous investigation (Winters, Smith and Mendel, 1927), it has been shown anew in the present study, that a ration distinctly deficient in inorganic salts alone, as is diet 2, induces cessation of growth in very young rats (21 days old) and that the more or less stationary level of body weight can be maintained throughout an experimental period of 40 days. During this time the "chronological" controls more than tripled their weight. The present study also confirms previous evidence that, in animals in which the increase in body weight is inhibited, there is a persistent augmentation in body and tail length with a consequent distortion of body form and of the body weight to body length ratio. Table 1 summarizes the data.

It is obvious that the normal rat at 21 days of age and the "low salt"

rat at 61 days of age are far more variable as regards body weight and body length measurements than is either the normal rat at 61 days of age or the "low calorie" rat 61 days old. This fact shows up in spite of the relatively small number of animals in the latter two groups. The considerable variation in tail length in all groups is also brought out in the comparison. The difference between the mean body length of the "low salt" group and that of the "physiological" controls is statistically significant $\left(\frac{D}{PED} = 6\right)$[4] and the same is true of the tail measurements $\left(\frac{D}{PED} = 25\right)$.

Bones. All determinations on bone were made on the right femur. At autopsy the shafts of the bones of the "low salt" group of rats were

TABLE 2

Moisture, ash and organic residue of femurs

GROUP	MEAN FRESH WEIGHT	MOISTURE	MEAN MOISTURE-FREE FAT-FREE, WEIGHT	ASH, ON MOISTURE-FREE, FAT-FREE, WEIGHT	A:R RATIO	COMPOSITION OF FRESH BONE			
						Water	"Fat"	Ash	Organic residue
	grams	*per cent*	*grams*	*per cent*		*per cent*	*per cent*	*per cent*	*per cent*
"Low Salt"	0.34±0.07*	56±4.5	0.13±0.02	34.8±2.8	0.53	56	6	13	25
"Physiol." Control.	0.28±0.02	60±0.4	0.09±0.01	44.7±0.5	0.80	60	7	14	18
"Chronol." Control	0.45±0.02	40±2.1	0.26±0.02	60.7±0.5	1.55	40	2	35	23

* Standard deviation.

noticeably fragile, frequently being fractured while handling the animal. The shafts of these bones were purplish-pink and gave the impression of being translucent. Winters, Smith and Mendel (1927) showed that in rats maintained for 40 days after weaning on a ration complete except for the low concentration of inorganic salts, the long bones continue to increase slowly in length though there is no increase in body weight. Furthermore, as is also shown in the present study, the fresh weight of the femurs of the "low salt" rats is greater than that of the "physiological" control group.

To what component of the bone is this increase due? The data bearing

[4] *D* is the actual difference between the means and *PED* is the probable error of the difference. The apparent difference between two means is significant when the quotient $\frac{D}{PED}$ is three or more. *PED* is the square root of the sum of the squares of the probable errors of the means (see H. C. Sherman, *Chemistry of food and nutrition*, 3rd ed., p. 604, Macmillan, 1926).

on this point obtained in the present investigation are summarized in table 2. The increase in fresh weight of the femur of the rats in the "low salt" group over that of the "physiological" control group is statistically significant $\left(\frac{D}{PED} = 5.3\right)$. The moisture content of the fresh bone of the "low salt" group is more like that of the infantile "physiological" control group than like that of the older "chronological" controls. There is, however, a significant difference between the values for the "low salt" and the "physiological" control groups $\left(\frac{D}{PED} = 5.97\right)$. The weight of the fat-free, moisture-free femurs of the "low salt" group was definitely greater than that of the "physiological" control group though only half that of the "chronological" controls. The per cent of ash calculated on the weight of the dry, extracted bone is strikingly different in the three groups, that of the "low salt" group being least (34.8 per cent), the "physiological" control group intermediate (44.7 per cent) and the "chronological" controls greatest (60.7 per cent). The greater weight of the dry, extracted bone of the "low salt" group over that of the "physiological" control group results in an absolute weight of ash in the bones of the two groups that is almost the same. When the content of the ash of the two groups is calculated as per cent of the fresh bone, the values are practiclly identical.

However, when the concentration of alcohol-ether insoluble organic matter of the bones of the stunted rats is compared with the same constituent in the bones of the "physiological" control group (with which they obviously should be compared), a striking difference is observed. The alcohol-ether insoluble organic matter accounts for a larger relative, as well as absolute, portion of the fresh bone in the "low salt" group than in that of the "physiological" controls. The per cent of this organic residue in the femurs of the stunted rats is even greater than that of the "chronological" control animals which have grown normally to more than three times the body weight of the "low salt" rats. This large proportion of organic matter in the bones is further emphasized by comparing the value of the A/R (ash to organic residue) ratio of the three groups of animals. Since normal growth of bone in the albino rat is characterized by an increase in percentage of organic residue (Hammett, 1925), this change in composition of the bones of the "low salt" group is still further evidence of the persistent tendency to grow in these animals.

From the available data it is apparent that when very young rats are given a ration deficient only in inorganic salts the bones are affected in the following manner: there is a tendency for the long bones to increase in weight and in length, the water content decreases less than is expected for the age, the ash concentration remains unchanged, i.e., the normal increase with age does not take place; but there is a striking increase in the

TABLE 3

Fresh weight, moisture and ash

GROUP	LEFT KIDNEY				SPLEEN			
	Mean fresh weight	Moisture	Ash	Comparison with "Chronol." control on basis of weight	Mean fresh weight	Moisture	Ash	Comparison with "Chronol." control on basis of weight
	grams	*per cent*	*per cent*		*grams*	*per cent*	*per cent*	
"Low Salt"	0.394±0.042*	78.0±2.8	1.4±0.2	75	0.057±0.017	79±1.6	1.8±0.04	15
"Physiol." Control	0.240±0.075	81.7±1.7	1.3±0.2	46	0.161±0.024	79±1.7	1.7±0.03	41
"Chronol." Control	0.528±0.039	76.8±1.3	1.1±0.1	100	0.388±0.080	78±1.0	1.3±0.03	100
"Low Calorie"	0.277±0.013	79.7±0.6	1.0±0.1	53	0.099±0.018	77±1.2	1.4±0.18	26

* Standard deviation.

alcohol-ether insoluble organic matter. It appears that this augmented organic residue accounts for the increase in length and weight of the femurs of the "low salt" group over similar measurements of the bones of the "physiological" controls. In the absence of the usual source of inorganic salts in the diet, all available minerals within the organism are husbanded for such indispensable functions as neutrality regulation, muscle and nerve activity and the maintenance of osmotic pressure relationships with the result that, although the pattern of the long bone is laid down in collagenous fibers and mucoid matrix, perfect calcification fails to take place and normal skeletal growth is allayed.

Kidney and spleen. One of the observations made by Winters, Smith and Mendel (1927) was the marked enlargement (persistent growth) of the kidneys of their "low salt" group of rats. Although the body weight of this group of animals remained the same as that of the "physiological" controls, the fresh weight of the kidney was much greater than that of the "physiological" control group. This observation has been confirmed in the present investigation, the fresh weight of the left kidney of the "low salt" rats being 63 per cent greater than the similar measurement in the "physiological" controls $\left(\frac{D}{PED} = 12\right)$.

To determine the cause of this increase, data on fresh weight, moisture and ash were obtained and are summarized in table 3. As might be expected, the moisture content of the kidneys of the "physiological" control group is greater than that of the "chronological" controls since the former animals are 40 days younger than the latter and relative dehydration is a normal accompaniment of age (Moulton, 1923). The "low salt" group resembles the older group in the water content of the kidneys $\left(\frac{D}{PED} = 2.6\right)$. The larger size of the kidney is therefore not due to an abnormal proportion of water. Whereas the percentage of ash in the kidneys of the "low salt" group is little more than that in the "physiological" control group $\left(\frac{D}{PED} = 1.5\right)$, the greater weight of the kidneys of the former animals results in an actual content of inorganic salts in the kidneys of the "low salt" group equal to that in the "chronological" controls, although the percentage of ash in the former is distinctly greater $\left(\frac{D}{PED} = 88\right)$. It has been demonstrated that the percentage of inorganic salts in the internal organs diminishes with advancing age (Hogan and Nierman, 1927). In the present study this expected decrease in mineral salt content of the kidney has not taken place in those rats receiving a strictly limited amount of ash in the diet but, instead, there has been a retention of mineral matter in the kidneys of these animals. This seems particularly signifi-

cant in view of the fact that simple underfeeding with an adequate ration has produced in the "low calorie" group a kidney whose ash relations are normal for its age (61 days).

This change in composition of the kidney is probably associated with the excretion of salts in the urine and may likely be dependent on the inorganic composition of the blood in these "low salt" animals. This point is under investigation at present. On the basis of the data thus far available it can be stated that the marked persistence of growth of the kidneys of the animals in the "low salt" group is accompanied by no other changes in composition than an increase in ash; it is due primarily to an actual increase of renal tissue.

The right kidney was used for histological examination. No abnormalities were observed in the kidneys of the "low salt" group of rats.

The small size of the spleen *immediately* after death in the rats of the "low salt" group was very noticeable. The fresh weight of this organ is definitely less than that of any other group, being even smaller than that of the "low calorie" rats. It is well known that stunting by underfeeding results in a decrease in the fresh weight of the spleen but it is shown in the present study that strict limitation of inorganic salts produces a still more pronounced decrement in size. The moisture percentage of the spleen as removed differs little among the groups. The relative quantity of ash in the spleens of the "low salt" rats is too high for the age of the animals but normal for the body weight. An interpretation of this observation must await a further study of the blood.

Blood. An enumeration of the red blood corpuscles at the end of the 40-day experimental period showed a striking increase of the red cell count in the animals of the "low salt" group. The data for the various groups of rats are summarized in table 4. The increase from 4.9 millions of the "physiological" controls to 6.3 millions of the "chronological" control rats is a normal accompaniment of growth in this animal. That inhibition of body growth *per se* is not responsible for the marked increase of red cells in the "low salt" rats is shown by the count of 6.4 millions in the blood of the "low calorie" group; this value is normal for the age of these animals (61 days).

In an effort to determine whether or not there is a correlation between the small size of spleen and the high erythrocyte count in the "low salt" rats, the spleen was removed from a fifth group of rats at weaning and they were then maintained on diet 2 for 40 days. The average red cell count in the eight animals surviving at autopsy was 10.6 millions, a difference from that of the intact "low salt" rats of doubtful significance. Therefore, the activity of the spleen does not account for the increase in red blood cells under the conditions of this experiment.

The observed increase in erythrocytes may be absolute or only apparent.

TABLE 4

Erythrocyte count, hemoglobin and size of red cells

GROUP	MEAN RED CELL COUNT	MEAN HEMOGLOBIN CONCENTRATION	RELATIVE RED CELL COUNT	RELATIVE HEMOGLOBIN CONCENTRATION	MEAN DIAMETER OF 500 RED CELLS	SIGNIFICANCE RATIO FOR RED CELL COUNT OF GROUPS $\frac{D}{PED}$	
	millions	*per cent*	*per cent*	*per cent*	*μ*		
"Low Salt"	11.1±1.3*	102±8.6	176	110	3.8	"Low Salt" / "Physiol." Control	37
"Physiol." Control	4.9±0.6	88±2.7	77	95	4.9	"Low Salt" / "Chronol." Control	28
"Chronol." Control	6.3±0.4	93±2.9	100	100	5.7	"Low Salt" / "Low Calorie"	26
"Low Calorie"	6.4±0.5		102				
"Low Salt Splenectomized"	10.6±0.8		168			"Low Salt" / "Low Salt Splenectomized"	2.0

* Standard Deviation.

If it is only relative, i.e., an evidence of change in blood concentration, and if no change in quality of the cells has taken place, the variations of hemoglobin concentration between the groups should parallel those of red cell count. That these variations are not parallel is obvious from table 4; the data indicate that the red corpuscles circulating in the blood of the animals of the "low salt" group are relatively deficient in hemoglobin.

It is plain, however, that the question of change in concentration of the blood cannot be settled on the basis of the relationships thus far discussed. A determination of both plasma protein concentration and total solids of the blood would permit one to draw a more definite conclusion on this point. Unfortunately, even after pooling the blood there was so little available at autopsy of the experimental rats weighing only 45 grams, that only a few determinations of plasma protein nitrogen were made for the various groups. The data suggest little if any difference in concentration of the blood in any of the four groups of animals which were 61 days old at the end of the experimental period. No determinations of total solids were made. Smith and Swanson (1929) have demonstrated that, in larger rats 130 days old, which have been maintained for 90 days on a similar ration poor in inorganic salts, there is a small though significant dilution of the blood as measured by the total solids and by plasma protein nitrogen. Although it has been shown that the very young rats used in the present investigation do not respond in all details as do the older animals used by Smith and Swanson under like dietary adjustment ("low salt"), the available data strongly suggest that the unusually high red cell count in the rats of the "low salt" group in the present study is not due to concentration of the blood.

Because of the limited quantity of blood available only a few determinations of red cell volume were made. Although these indicate a small increase in cell volume in the blood of the "low salt" group, the value is very much less than would be expected for the increase in number of erythrocytes in the blood of this group. This observation led to the measurement of the diameter of the red corpuscles of the blood of the various groups. The data in table 4 show that the erythrocytes in the rats of the "low salt" group are even smaller than those of the "physiological" control animals. The evidence leads to the conclusion that, as a result of strict limitation of inorganic salts in the ration of very young rats, there occurs after 40 days a marked overproduction of under-sized red blood corpuscles unaccompanied by changes in concentration other than those normally incident to growth.

The changes observed in certain organs and blood constituents are striking; it is plain, however, that before any explanation for these alterations can be given, a study of metabolism and change of distribution of salts throughout the body must be made under the dietary régime em-

ployed. It is impossible to obtain satisfactory samples of blood and urine from rats weighing only 45 grams. An attempt has been made, therefore, to reproduce the condition in larger animals under similar dietary adjustment and a preliminary report on these studies has already been made (Smith and Swanson, 1929).

SUMMARY

Young rats maintained for 40 days on an experimental ration deficient only in ash do not increase in body weight. The body and tail lengths of such rats at the end of the experimental period (40 days) are greater than those of normal rats of the same body weight. The leg bones are heavier than those of normal rats of the same weight, the increase being largely accounted for by the higher content of organic residue. The fresh weight of the kidneys of the experimental animals is strikingly greater than that of normal rats of the same weight. The concentration of moisture, ash and organic solids of the kidney is similar to that of normal rats of the same weight rather than age. The fresh weight of the spleen is diminished and this organ is more like that of the normal weight controls than age controls. There is a polycythemia, the cells being smaller than normal and the total hemoglobin of the blood less than normal.

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CONDITIONS AFFECTING THE LINGUO-MAXILLARY REFLEX

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The linguo-maxillary reflex was first described by Cardot and Laugier (1922) in the dog. It consists of a mouth-opening movement on electrical stimulation of the mucous membrane of the tongue. The muscles involved are the supra-hyoid group, particularly that muscle which corresponds to the digastric in man. In the dog this muscle has only one belly; it arises from the jugular process of the temporal bone and is inserted on the medial aspect of the mandible (Ellenberger und Baum, 1891). However, all the muscles of the anterior face of the neck are involved in the response. The afferent path of the reflex arc is through the lingual branch of the trigeminus and the efferent through the mylo-hyoid branch of the mandibular.

Cardot and Laugier were particularly interested in the effect of the depth of anesthesia on the magnitude of the reflex. They made a quantitative study of the threshold current necessary for the elicitation of the reflex in the dog when chloroform-alcohol, chloralose, and chloral-morphine were used as anesthetics, and found that the threshold varied directly with the degree of anesthesia. Brocq-Roussen, Cardot and Laugier (1923) made similar observations on the horse, the cat and the rabbit. In all these animals, as the depth of anesthesia increased, the linguo-maxillary reflex persisted longer than did the corneal reflex, or the labio-mental reflex of Dastre. The authors suggest that the linguo-maxillary reflex be termed the ultimate reflex, and they advise its use in man as a criterion of the depth of anesthesia.

Cherbouliez and Laugier (1926) stimulated the vagus nerve while eliciting the linguo-maxillary reflex in the dog. There resulted an immediate and sustained drop in blood pressure and an augmentation of the reflex response. On cessation of the vagus stimulation the blood pressure regained its former level and the reflex subsided to somewhat less than its original magnitude. These results were held to demonstrate the extreme sensibility of the central nervous system to anemia, the reflex centers being first excited and later depressed if the blood supply is decreased for too great a period of time.

Our experiments were performed on dogs. The animals were usually

anesthetized with barbital sodium. Blood pressure was recorded from the carotid artery, and a respiration tracing was made by means of a pneumograph and tambour. The digastric muscle on one side was dissected and its tendinous attachment, freed from the mandible, was connected by a thread to a tambour which communicated with a recording tambour by means of rigid rubber tubing. When the muscle contracted the air was forced out of the first tambour into the second, the upstroke of the writing lever of the recording tambour thus indicating contraction of the muscle. This method of recording, which we adopted from Laugier, surpasses in sensitiveness any mechanical device employing a system of pulleys and levers. Two artery clips connected by wires to the secondary coil of an inductorium, clamped on to the tongue of the dog, served as stimulating electrodes. In the primary circuit there was inserted a clock-work device which made and broke the circuit automatically and continually, the frequency most often employed varying from 150 to 200 per minute. To eliminate injury to the muscle on account of exposure to the air, the skin incision was made as short as possible and the small portion of the muscle that had to be left exposed was covered with cotton. The head of the animal was securely immobilized in a head-holder, to make sure that no other movements, aside from the contraction of the digastric muscle, were recorded.

The effect of cephalic anemia. Our chief interest in the previous work on the linguo-maxillary reflex lay in the experiment of Cherbouliez and Laugier on the influence of anemia on the medullary centers. Like these authors we repeatedly stimulated the peripheral end of the cut vagus nerve, while eliciting the linguo-maxillary reflex, but we obtained no augmentation of the reflex nor a sustained drop in blood pressure. The heart always broke through the vagus inhibition, and we ascribed our failure to observe an increase in the reflex to the incompleteness of the anemia. In some experiments chloroform or chloral hydrate was used as an anesthetic because of the cardiac toxicity of these drugs, with no better results. Negative results were also obtained with the use of other anesthetics, such as ether and paraldehyde. In a series of 25 dogs we encountered only one animal that showed an augmentation of the linguo-maxillary reflex as a result of peripheral vagus stimulation. That animal was anesthetized with barbital sodium, but unlike the effect in other dogs under the same anesthetic, stimulation of the vagus produced a sustained fall of blood pressure in this dog.

Cherbouliez and Laugier did not indicate the anesthetic that they used in their experiments. We have learned recently that they employed chloralose, and under its influence, vagus stimulation always produced a sustained inhibition of the heart and a concomitant augmentation of the reflex. It seemed then that a suddenly produced and long maintained anemia was a

condition necessary for the excitation of the medullary centers preceding their subsequent depression. To test this provisional conclusion we em-

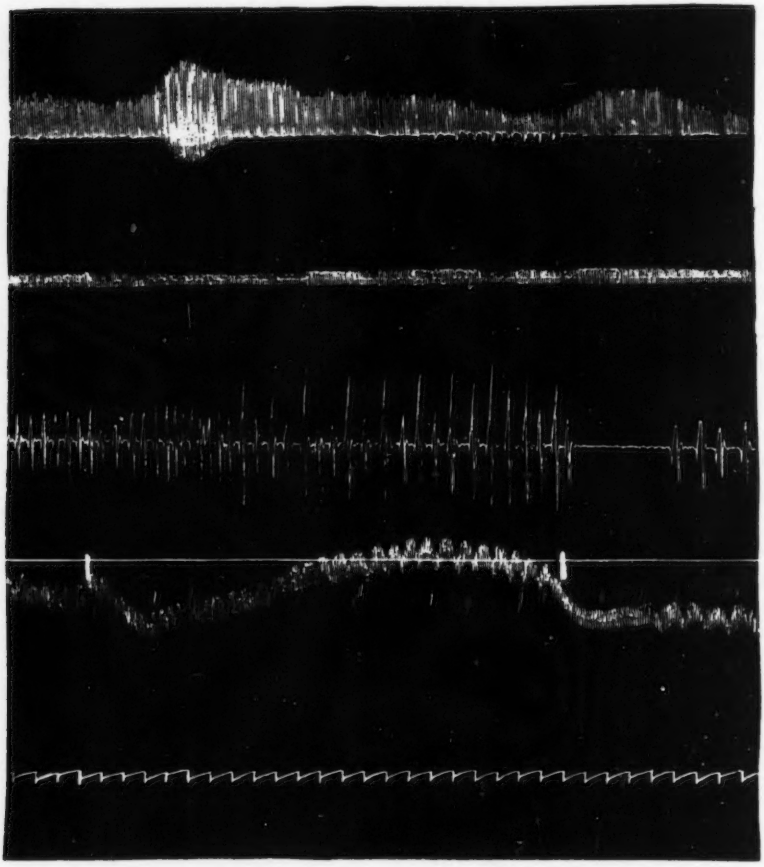


Fig. 1. The effect of occlusion of cephalic blood vessels upon the magnitude of the linguo-maxillary reflex. The records represent from above downward: reflex contractions of the digastric muscle, signal magnet indicating stimulations of the tongue, respiration, signal magnet indicating beginning and end of cephalic anemia, blood pressure, and 5 seconds time record serving as base line for blood pressure.

ployed two other methods of producing a sudden and sustained cephalic anemia.

The first of these consisted of a sudden occlusion of the arteries carrying

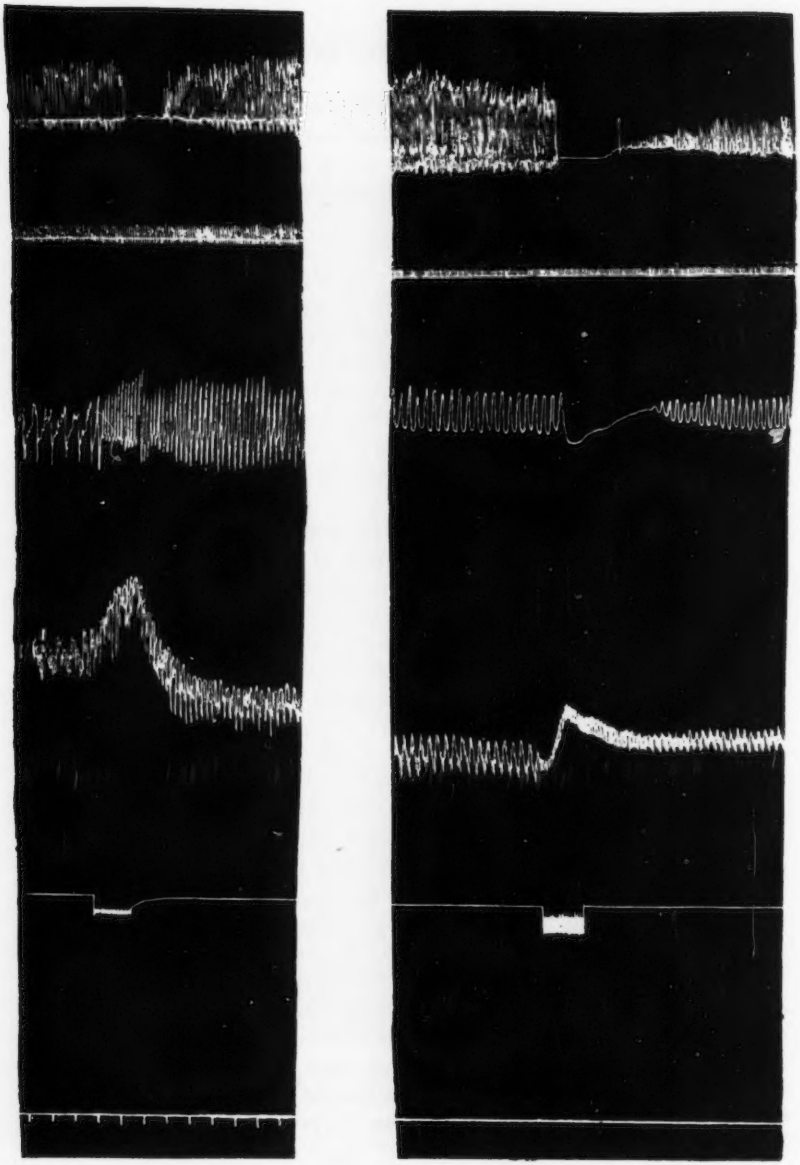
blood to the head. At the beginning of the experiment the two vertebral arteries were tied off. Blood pressure was recorded from one of the femoral arteries. By compression of the two carotid arteries a marked degree of cephalic anemia could be produced, the only source of blood supply to the medulla being the small spinal arteries.

The production of cephalic anemia by this method led to an immediate rise in blood pressure, occasionally accompanied by a change in the character of the respiration. That these effects were not due to a decrease in the size of the vascular bed was demonstrated by the fact that simultaneous occlusion of one subclavian and one femoral artery had no such action. Again, as with the stimulation of the peripheral end of the cut vagus, in only one dog could we produce a marked increase in the magnitude of the reflex by clamping off the carotids. In this dog the blood pressure was very low (fig. 1). We clamped the carotids three times with the same results.

The other method of producing cephalic anemia was adopted from Johnson and Luckhardt (1927), and it consisted of a forcible distention of the lungs by inflation through the trachea, followed by the clamping of the trachea. This produces an immediate and very marked drop in the blood pressure, since the veins are collapsed and no blood can enter into the chest cavity. We applied this method to almost all the dogs studied, and it generally led to a gradual disappearance of the reflex response. When the animal was allowed to breathe again, the reflex gradually returned to normal. Here, too, in only one dog, under light ether anesthesia, did overdistention of the lungs lead to an increase in the reflex response, but this was preceded as well as followed by a complete inhibition of the reflex.

The effect of afferent stimuli. Although we were unsuccessful in our efforts to produce a regular augmentation of the linguo-maxillary reflex, its inhibition could be brought about with great ease. Stimulation of the central end of the cut vagus nerve caused in practically every case a complete abolition of the reflex, lasting as long as the stimulation was continued (fig. 2). On cessation of the stimulation the response gradually returned to its former height. Stimulation of the intact nerve had the same effect, while stimulation of the peripheral end of the cut vagus, as already indicated, was without influence upon the reflex. Stimulation of the intact sciatic nerve or the central stump of the cut nerve also resulted in complete abolition of the reflex during the period of stimulation (fig. 2). Digital distention of the rectum and mechanical or electrical stimulation of the viscera caused a depression of the reflex, but never a complete abolition. When chloroform was used as an anesthetic, the recovery of the reflex upon cessation of stimulation was very slow.

Several experiments were performed in an endeavor to analyze the mechanism of the inhibition of the reflex by stimulation of the sciatic.



A B

Fig. 2. Inhibition of the linguo-maxillary by the stimulation of the central end of the cut sciatic nerve (A) and of the central end of the cut vagus nerve (B). The records represent from above downward: reflex contractions of the digastric muscle, signal magnet indicating stimulations of the tongue, respiration, blood pressure, signal magnet indicating duration of nerve stimulation, blood pressure base line, indicating 5 seconds' intervals of time.

If stimulation of the sciatic nerve could be shown to result in a closure of the mouth the inhibition of the reflex opening of the mouth could be explained on the principle of reciprocal innervation. But on several occasions, keeping the mouth artificially opened and using the jaw as a whole or the isolated temporal or masseter muscle for recording, we could observe no such effects upon stimulation of the sciatic nerve.

Fatiguability of the linguo-maxillary reflex. We were impressed by the fact that in some cases, under moderate narcosis, the reflex gave no indications of fatigue. Where fatigue was in evidence, a short time sufficed to produce complete recovery. Resistance to fatigue was especially marked in dogs that appeared to be in very good physical condition. In an attempt to produce fatigue in such a dog more than 90,000 successive stimulations were applied (at the rate of 200 per minute) and the magnitude of the response remained practically unchanged throughout the several hours of stimulation.

In deeper anesthesia the reflex tended to disappear. As alluded to previously, its discoverers termed it the "ultimate reflex," but we found that this was only true as far as the corneal reflex was concerned. The knee jerk always outlasted the linguo-maxillary reflex when the anesthesia became very deep. Doctor Luckhardt also found that after over-distention of the lungs the linguo-maxillary reflex was abolished whereas the knee jerk could still be obtained.

Palato-maxillary reflex. On one occasion, as a result of electrical stimulation of the soft palate, a forcible closing of the mouth was observed. Stimulation of the hard palate called forth a reflex opening of the mouth, similar to that obtained by stimulation of the tongue. If the electrodes were placed farther back, the mouth opening movement changed to a closing one, and this latter became more and more intense as one approached the posterior end of the soft palate. An intermediate position could sometimes be found where stimulation gave a combination of both effects, an oscillatory movement of the jaw.

Direct observation revealed several interesting facts in connection with the mouth-closing movements resulting from the stimulation of the soft palate, which we propose to call the *palato-maxillary reflex.* In addition to the contraction of the temporal and masseter muscles closing the mouth, the digastric, which tends to open the mouth, also contracts. The contractions of all the muscles take place simultaneously, but since the mouth closers have the mechanical advantage, the result is a closing of the mouth. It would appear that we are dealing with two antagonistic reflexes elicited simultaneously.

Unlike the linguo-maxillary reflex, the palato-maxillary response is not affected in any way by stimulation of the central end of the sciatic nerve or of the vagus.

SUMMARY

1. Cephalic anemia produced in anesthetized dogs by *a*, peripheral vagus stimulation; *b*, pulmonary inflation, and *c*, occlusion of the cephalic blood vessels, does not produce a consistent augmentation of the linguo-maxillary reflex.

2. Stimulation of the central end of the cut sciatic nerve, or the vagus, or of visceral afferent fibers, causes an immediate abolition of the linguo-maxillary reflex, with a quick return to normal upon cessation of the stimulation.

3. The resistance to fatigue of the linguo-maxillary reflex varies with individual dogs. In one animal 90,000 successive stimulations were insufficient to produce any evidence of fatigue.

4. The linguo-maxillary reflex is not "ultimate," as in progressive depression of the central nervous system it disappears before the knee jerk.

5. A new reflex, closure of the mouth upon electrical stimulation of the soft palate, is described. We designated it as the palato-maxillary reflex.

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A PLETHYSMOGRAPHIC STUDY OF THE THYROID GLAND OF THE DOG

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During the last several years collodion plethysmographs of the type described by Hargis (1925–1926) have been employed in our laboratory for measuring changes in volume of various viscera. Because of the ease with which a plethysmograph can be applied to the thyroid gland we have deemed it wise to investigate systematically the changes in volume manifested by this gland under varying conditions. In the study reported in this paper the experiments were all of an acute nature; the plethysmograph was applied under ether anesthesia, and the animal was killed at the end of the experiment.

METHOD. The construction of the plethysmographs in general is the same as that for other organs. A series of these plethysmographs should be kept on hand since the size of the thyroid gland in dogs from this section of the country is unpredictable. The method of recording changes in the volume of such a relatively small organ as the thyroid gland necessitated the use of the piston recorder of Lombard and Pillsbury (1899–1900). This instrument was modified slightly, since it was a little too delicate for our purpose. Thus the diameter of the piston was increased to 6 mm. and the writing arm was heavier than the specifications of Lombard and Pillsbury.

After the plethysmograph had been adjusted around the thyroid gland it was found essential to approximate the tissues at both ends of the gland around the plethysmograph by means of linen sutures. In recording blood pressures, the cannula was inserted as a routine into the opposite carotid artery (fig. 1).

THE EFECT OF CERTAIN DRUGS. The effect of drugs on the volume of the thyroid gland has been reported by several observers who have used various methods of investigation. Gunning (1917) inserted cannulas into the inferior thyroid veins of dogs, recorded the femoral blood pressure, and gave epinephrine intravenously in pressor, depressor and threshold doses. He recorded, on a smoked drum, the variations in the number of drops of blood from the veins and found that epineph-

rine caused marked diminution in flow of blood through the thyroid gland. He concluded that epinephrine caused the same effects as those caused by stimulation of the cervical sympathetic nerves. He stated that following administration of epinephrine there was over-recovery of the gland. He also stated that he had never observed primary dilation of the gland following administration of epinephrine. Schkawera and Kotschergin (1925) removed the thyroid glands of several different animals, and perfused them through the superior thyroid arteries, with Ringer-

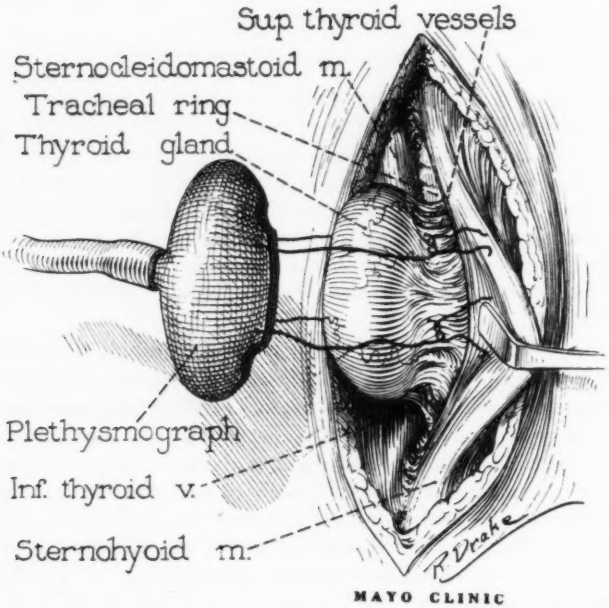


Fig. 1. Method of applying the plethysmograph to the thyroid gland of a dog. After it is thus applied the fascial structures surrounding it are sewn over it to make it air-tight.

Locke's solution to which certain drugs, in physiologic dosage, had been added. They counted the number of drops coming from the inferior thyroid vein in a given period of time. They found that the addition of epinephrine to the perfusing fluid caused a weak vasoconstrictor action, whereas caffein had a dilator effect. Engel (1926) investigated the effect of epinephrine on the intact thyroid gland. He stated that when a solution of epinephrine 1:1000 was applied locally to one lobe, while the opposite lobe was used as a control, there was marked congestion of the portion of the gland to which it was applied. He also reported that following the

intravenous injection of 4 cc. of epinephrine 1:1000, in the dog, there was marked bilateral engorgement which persisted for about five minutes, after which the gland shrunk. He concluded that the action was specific to the thyroid vessels, and was not due to the general effect of the intravenous injections on the circulation as a whole. The experiments can be severely criticized on the grounds that the dosages employed were too large to be physiologic.

Cannon and Cattell (1916) noted that there was an action current in the thyroid gland of a cat following the intravenous injection of 0.1 cc. of epinephrine 1:100,000. They interpreted this as indicating that the thoracic autonomic apparatus was secretory to the thyroid gland. This conclusion was not without merit, since action currents have been reported by Bayliss and Bradford (quoted by Cannon and Cattell) during the secretion of saliva by the submaxillary gland.

It is necessary to emphasize that the results which are here reported are not dependent on alterations of one sort or another in the surrounding structures of the neck, since control experiments have been performed in which the thyroid vessels were ligated. Also mock experiments, in which the empty plethysmograph was connected as usual to the piston recorder, and was placed in the neck of the dog, have given negative results. A change, which is not dependent on volume of the thyroid gland, occurs in consequence of reflex swallowing, when the anesthesia is too superficial. Reflex tonic spasm of the ribbon muscles of the neck is easily recognized. The fact that the plethysmographs for use on the thyroid gland are rigidly constructed, and that curarizing the animals makes no difference in the results obtained, is good evidence that the results are due, indeed, to alterations in the volume of the thyroid gland, in spite of the fact that such alterations are often quite marked.

Results. The intravenous injection of the following drugs brought about the usual changes of blood pressure and corresponding changes in the volume of the thyroid gland (fig. 2): nicotine, pilocarpine, nitroglycerine, acetyl choline, atropine, pituitrin, and rattlesnake venom. The intravenous injection of 0.1 to 0.2 cc. of epinephrine 1:1000 to a dog of average size usually evoked marked shrinkage in volume of the thyroid gland, often with preliminary dilatation preceding the fall in volume. There was often an expansile after-effect. In about a fourth of the experiments, primary injection of epinephrine in the foregoing dosage was followed by definite increase in volume of the thyroid gland. The action of epinephrine was so definite that it was adopted as a routine for beginning and closing an experiment, as a criterion that the apparatus was in working order.

Thyroid extract, in aqueous solution (0.325 gm. given intravenously) produced a fall in volume of the thyroid gland with but slight change in blood pressure (fig. 2*k*). Thyroxine was injected in alkaline solution

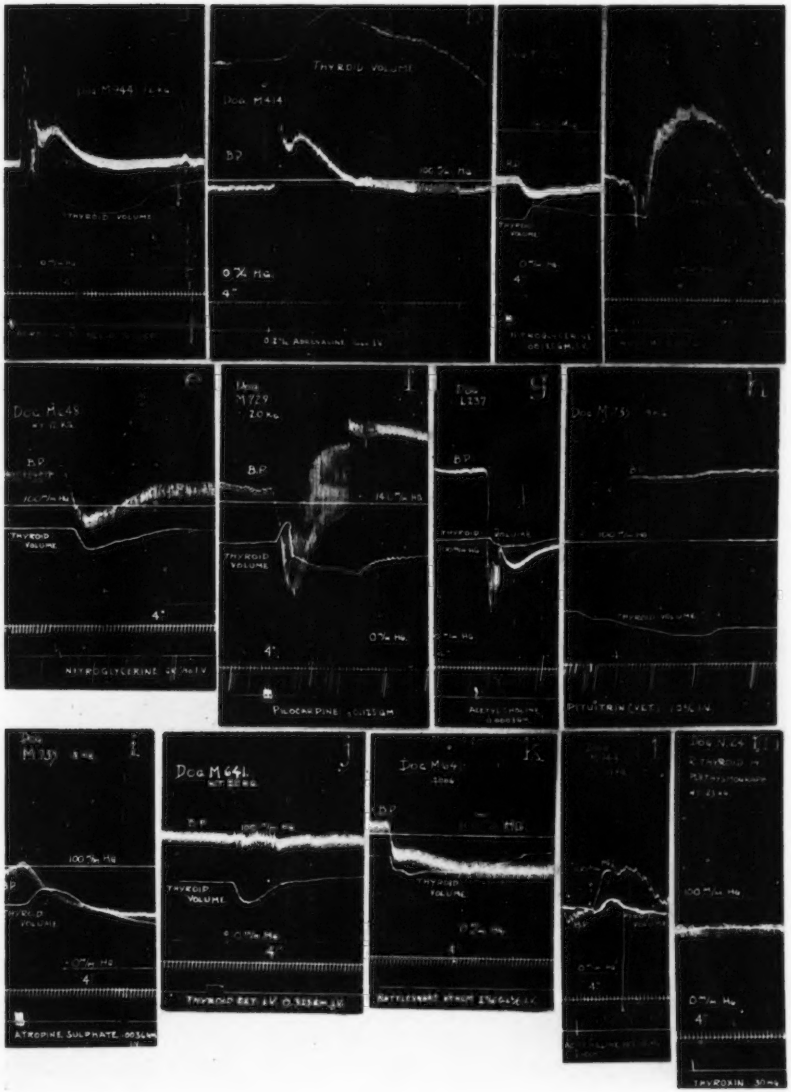
(freshly prepared) in doses of 3 mgm., 10 mgm., and 30 mgm. without effect either on volume of the thyroid gland or on blood pressure (fig. 2*m*).

Physiologic doses of strophanthin, potassium iodide, and caffein sodium benzoate have little, if any, effect on volume of the thyroid gland.

Comment. These experiments have not resulted in anything unusual as regards the action of drugs on the volume of the thyroid gland; the changes here recorded, in all instances, are explicable on a circulatory basis. The fact that thyroid extract caused shrinkage cannot be regarded as significant, since in the absence of any reaction with thyroxine, this result must be explained as a non-specific response to tissue extract. The fact that epinephrine, in certain experiments, evoked expansion of the thyroid gland cannot be regarded as evidence of an extraordinary reaction of the thyroid gland to stimulation of the thoracic autonomic apparatus. This result is more logically interpreted on the basis of a passive shift of the blood to the periphery in consequence of splanchnic vasoconstriction. It must be borne in mind, in this connection, that the normal response of skeletal muscle to epinephrine is expansion.

THE EFFECT OF STIMULATION OF NERVES. This phase of the plethysmographic study of the thyroid gland was undertaken to determine the effect on volume of the gland of electrical stimulation of the vagus and sympathetic nerves of the gland. Since there has been much speculation regarding the nature of the nerve supply to this organ and since the existence of a secretory nerve of the thyroid gland has been debated it appears advisable to review briefly some of the pertinent literature in this regard. Asher and Cannon and their associates have been outstanding in this work. Asher and Flack found, when they stimulated the superior and inferior

Fig. 2. *a*, epinephrine producing rise in blood pressure with fall in volume of the thyroid gland, and the later over-recovery; *b*, epinephrine producing increase in volume of the thyroid gland, and the return to normal; *c*, nitroglycerine producing fall in blood pressure and increase in volume of the thyroid gland; *d*, nicotine producing rise in volume of the thyroid gland paralleling blood pressure; *e*, nitroglycerine producing fall in both blood pressure and volume of the thyroid gland; *f*, pilocarpine producing preliminary rise, followed by shrinkage of volume of the thyroid gland; *g*, acetyl choline producing fall in blood pressure and paralleling action on volume of the thyroid gland; *h*, pituitrin producing a slow, prolonged rise in blood pressure and slow, prolonged shrinkage of volume of the thyroid gland; *i*, atropine sulphate producing a slight rise in blood pressure followed by a fall, and increase in volume of the thyroid gland, followed by fall; *j*, rattlesnake venom (*Crotalus atrox*) producing maintained fall in blood pressure; volume of the thyroid gland shows a fall, with slow rise above level of injection; *k*, thyroid extract producing fall in volume of the thyroid gland without appreciable change in blood pressure; *l*, action of epinephrine after nicotine, giving increase in volume of the thyroid gland and usual effect on blood pressure. This action is reversal of that of epinephrine in the same animal, *m*, thyroxine producing no effects on blood pressure or volume of the thyroid gland after the injection of 30 mgm. of this substance.


THYROID VOLUME
Dog M414
B.P.
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THYROID VOLUME
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EPITHELIAL EXTRACT
THYROXIN

Fig. 2

laryngeal nerves before they had stimulated the depressor nerve,that there was a greater fall in blood pressure than was ordinarily seen. Under similar circumstances, the injection of epinephrine raised the blood pressure to a greater height than before. The same phenomenon occurred after the intravenous injection of thyroid extract. Therefore, these authors concluded that the laryngeal nerves were secretory to the thyroid gland. The following year Biedl and Brund confirmed the observation that stimulation of the depressor nerve evoked a greater fall in blood pressure after the laryngeal nerves had been stimulated. However, these experiments do not fix the responsibility for nervous control of thyroid secretion, since the laryngeal nerves contain both thoracic autonomic, and craniosacral fibers.

Several workers, by sectioning nerves, hoped to obtain data on the secretory control of the thyroid gland. The histologic appearance of the gland following degenerative section of the vagus and sympathetic nerves yielded conflicting results (Misseroli, 1908–1909; Wiener, 1909; Schilf and Heinrich, 1924; Crawford and Hartley, 1925). A comparison between the iodine content of opposite lobes has apparently yielded the result that stimulating the superior cervical trunk diminishes the iodine content on the same side (Rahe, Rogers, Fawcett, and Beebe, 1914; Watts, 1915). By means of precipitin methods for the estimation of thyroglobulin in dogs Hektoen, Carlson and Schulhof (1927) established that there was no increase of thyroglobulin in specimens of blood coming from the gland following stimulation of the cervical sympathetic nerve. Hicks (1926–1927) confirmed this result.

Cannon and his pupils (Cannon and Cattell, 1916; Cannon and Smith, 1920) obtained evidence which indicated that the sympathetic trunk might be the secretory nerve to the thyroid gland. They studied the variations in the electrical potential of the thyroid gland which occurred when the sympathetic trunk in cats was stimulated immediately below the stellate ganglion.

Asher and Pflüger (1928) following a study of the speed of resorption of an artificially established exudate or edema concluded that the cervical sympathetic trunk was secretory to the thyroid gland.

Experimental methods. In this study, the vagosympathetic trunks on either side were isolated low in the neck, and linen loops were placed about them. The plethysmograph was applied to the selected lobe of the thyroid gland, and the blood pressure in the carotid artery of the opposite side was taken. A preliminary dose of epinephrine was given to determine whether the apparatus was functioning smoothly. The vagosympathetic trunks were then tied and cut. Faradic stimulation was applied to the central ends of the cut nerves. In certain experiments, these nerves were stimulated in the thorax. In these cases, the vagus and sympathetic nerves were exposed proximal to their union. Intratracheal insufflation was employed in these experiments.

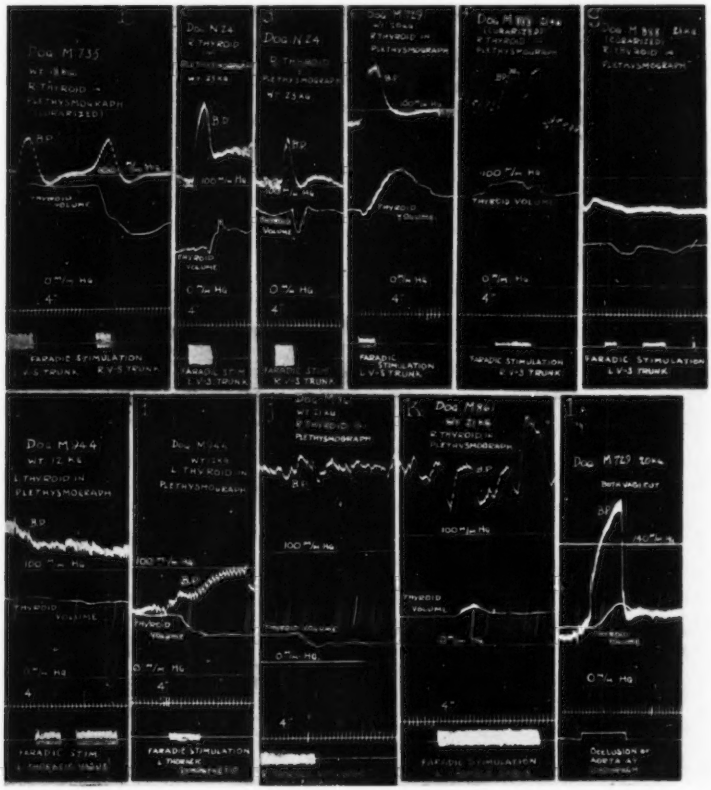

Fig. 3. *a*, stimulation of contralateral vagosympathetic trunk with rise both in volume of the thyroid gland and blood pressure; *b*, stimulation of homolateral vagosympathetic trunk with fall in volume of the thyroid gland and rise in blood pressure; *c*, stimulation of contralateral vagosympathetic trunk, with rise in both volume of the thyroid gland and blood pressure; *d*, stimulation of homolateral vagosympathetic trunk, with fall in volume of the thyroid gland and rise in blood pressure; *e*, stimulation of contralateral vagosympathetic trunk, with rise both in volume of the thyroid gland and blood pressure; *f*, stimulation of homolateral vagosympathetic trunk with rise in volume of the thyroid gland and blood pressure; *g*, stimulation of contralateral vagosympathetic trunk, with fall in volume of the thyroid gland; no rise in blood pressure; *h*, negative result from stimulation of homolateral vagus nerve in the thorax; *i*, stimulation of homolateral thoracic sympathetic with fall in volume of the thyroid gland and rise in blood pressure; *j*, stimulation of homolateral thoracic sympathetic, with fall in volume of the thyroid gland and rise in blood pressure; *k*, stimulation of homolateral vagus nerve in the thorax, with rise in volume of the thyroid gland and in blood pressure, *l*, rise in volume of the thyroid gland and in blood pressure following temporary occlusion of the aorta at the diaphragm.

In several instances the etherized dogs were curarized to obviate reflex movements of the neck and pharyngeal muscles. As in previous experiments, these curarized preparations gave results that were identical with the non-curarized animals.

RESULTS. The cephalic end of the vagosympathetic trunks, when stimulated by a weak tetanizing current, almost invariably produced the following results. On stimulation of the homolateral trunk, with the exception of two experiments, there was a sharp decrease in volume of the thyroid gland. The decrease was similar in type to that produced by epinephrine (figs. 3 *b* and *d*). The exception to the foregoing was an increase when the homolateral vagosympathetic trunk was stimulated (fig. 3 *f*) or else no change in volume from this procedure (fig. 3 *h*). Stimulation of the cephalic end of the opposite vagosympathetic trunk usually produced an increase in volume of the thyroid gland (figs. 3 *a*, *c* and *e*). The exception to this rule was a decrease (fig. 3 *g*). Uniformly, the increase in volume accompanying stimulation of the contralateral vagosympathetic trunks was accompanied by the usual rise in blood pressure. When the blood pressure failed to rise, there was no change in the volume of the gland. That stimulation in these cases was effective was evident from the characteristic changes in respiration. The shrinkage in volume or increase in volume was not abolished by preliminary atropinization.

Stimulation of the homolateral sympathetic trunk, proximal or distal to the stellate ganglion in the thorax, usually gave a decrease in volume of the thyroid gland (figs. 3 *i* and *j*), whereas stimulation of the opposite thoracic sympathetic trunk produced an increase in volume. Stimulation of the nerve in the thorax usually was without effect; in one instance, however, stimulation of the homolateral vagus nerve in the thorax was accompanied by an increase of thyroid volume (fig. 3 *k*).

Comment. The experiments described cannot in any manner be used as evidence that the thyroid gland possesses a secretory nerve; however, they have definitely indicated that the sympathetic portion of the vagosympathetic trunk is vasoconstrictor to the vessels of the thyroid gland. Although in one experiment there was a rise following stimulation of the homolateral vagosympathetic trunk, this increase was accompanied by a prolonged increase of the blood pressure, so that this expansion probably was secondary.

This is true, also, of the increase in volume following stimulation of the homolateral vagus nerve in the thorax before it is joined by the sympathetic trunk.

Stimulation of the recurrent laryngeal nerve had no influence on the volume of the thyroid gland.

That the volume of the thyroid gland responds passively to changes in blood pressure has been readily shown by compressing the aorta at the

diaphragm, giving a sharp rise in both blood pressure and volume of the thyroid gland (fig. 3 *l*).

SUMMARY

A method of plethysmography of the thyroid gland has been described.

The effects of various drugs on the volume of the thyroid gland have been studied. The effect of the following drugs is explicable on a circulatory basis: epinephrine, nicotine, nitroglycerine, pilocarpine, acetyl choline, atropine, pituitrin, rattlesnake venom, thyroid extract, and thyroxine. The following drugs had little, if any, effect: strophanthin, potassium iodide, and caffein sodium benzoate.

The effects on volume of the thyroid gland are recorded following stimulation of the vagus and sympathetic nerves in the neck and thorax. These experiments have demonstrated that the vasoconstrictor fibers pass to the thyroid gland by way of the sympathetic nerves. Following stimulation of the homolateral vagus nerve in the thorax, before its juncture with the sympathetic trunk, or following stimulation of the opposite vagosympathetic trunk, there is produced an increase in the volume of the thyroid gland. This was considered to be secondary to increased flow of blood consequent on the concomitant rise in blood pressure. In the researches recorded, the opportunity often presented itself for studying the reactions of hypertrophied glands to the stimuli that have been mentioned. The results were identical with what occurred in non-goitrous animals. As might be expected, changes in volume were correspondingly greater in large goitrous glands. In several instances our experiments were repeated on animals that had been rendered hyperthyroid by injecting thyroxine, or by feeding desiccated thyroid gland. The results were identical, whether one substance or the other was used.

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THE EFFECT OF ADRENALIN ON THE AURICLE OF ELASMOBRANCH FISHES

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Since the early work of Oliver and Schäfer (1895) who found augmentation and acceleration of the mammalian heart on injection of suprarenal extract after section of both vagi, much has been written on the effect of adrenalin on the hearts of various vertebrates. The only work on the effect of adrenalin on the fish heart is by Macdonald (1925) who perfused the heart of the dog-fish, Scyllium, with a solution of adrenalin and obtained a preliminary inhibition of rate with a subsequent increase in amplitude and sometimes acceleration.

The work reported in the present paper was undertaken for several reasons. First, Macdonald's results do not fall readily into line with the well-known effects of adrenalin and of stimulation of the sympathetic nervous system on the heart of other vertebrates. Second, the sympathomimetic effect of adrenalin has some exceptions, notably in the case of the pulmonary circulation in the frog (Luckhardt and Carlson, 1921) and the sweat glands (Langley, 1921). Third, adrenalin has an accelerating effect on the heart of certain invertebrates (Hogben and Hobson, 1924; Bain, 1929) where no sympathetic innervation has been described. Fourth, there is some evidence that when means are taken to lower the threshold of the parasympathetic or paralyze the sympathetic supply to the heart, adrenalin causes inhibition which is released by atropine (Amsler, 1920; Kolm and Pick, 1921; and Heinecamp, 1926). The work of these authors suggests that the site of action of adrenalin depends upon the threshold balance of the parasympathetic and the sympathetic nerves. Fifth, elasmobranch fishes have a well-developed chromaphil system, extracts of which give characteristic adrenalin effects (Lutz and Wyman, 1927). If adrenalin is sympatho-mimetic, or acts only on sympathetic endings, one might expect an altered effect on the elasmobranch heart, which probably is not supplied with accelerator nerves (Bottazzi, 1902; Müller and Liljestrand, 1918; Lutz, 1930a). In this heart there is a well developed parasympathetic supply without an equally balancing sympathetic innervation. Reasoning by analogy the product of the chromaphil tissue might

be expected to produce an effect similar to the sympathetic if it were present, that is, acceleration of the heart. However, if the site of action of adrenalin depends on the relative parasympathetic-sympathetic threshold, inhibition might be expected unless it is obscured by a direct effect on the heart muscle.

MATERIALS AND METHOD. The elasmobranch fishes used were three species of skate, namely, Raia erinacea, R. diaphanes, and R. scabrata, and the dogfish Squalus acanthias. An isolated sinus-auricle preparation was used in order to avoid possible disturbing effects of auriculo-ventricular block and reversal of the beat, both of which occur easily when the elasmobranch heart is perfused and the record taken from the ventricle. The auricle was suspended in an immersion fluid by two small hooks and two threads;

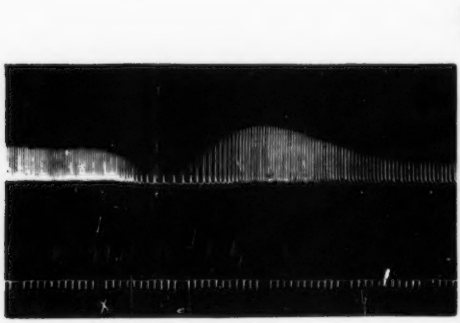

Fig. 1

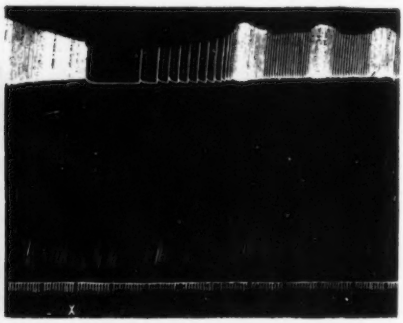

Fig. 2

Fig. 1. Raia erinacea. Sinus-auricle preparation. Adrenalin chloride added at *X* to make 1 in 12,500. Time in 5 second intervals.

Fig. 2. Squalus acanthias. Sinus-auricle preparation. Adrenalin chloride added at *X* to make 1 in 50,000.

one attached to an L-shaped rod below and the other to a counterbalanced writing lever above. The composition of the immersion fluid was based on the analysis of elasmobranch blood made by Dr. Homer W. Smith at this station. Each liter contained sodium chloride, 16.38 grams; potassium chloride, 0.596 gram; calcium chloride, 0.555 gram; sodium bicarbonate, 0.168 gram; and urea, 21.6 grams. The pH was 7.5. Adrenalin chloride (Parke, Davis & Company) was added directly to the fluid (30–40 cc.) by means of a pipette. This was mixed gently but thoroughly without disturbing the preparation mechanically. Inasmuch as adrenalin chloride contains slightly less than 0.5 per cent chloretone, it was considered necessary to control with equivalent doses of 0.5 per cent chloretone solution. The amount of adrenalin used did not lower the pH more than 0.4, and Macdonald (1925) has shown that such large variations from pH 7.0 had little effect on the elasmobranch heart.

RESULTS. The typical effect of adrenalin chloride on the auricle was a temporary slowing or even a complete inhibition, and a decrease in amplitude followed by recovery of both rate and amplitude, the latter sometimes becoming greater than before. In one case there was an increase in rate during the recovery period over the original. Adrenalin chloride added to the immersion fluid to make solutions of 1 in 50,000 to 1 in 25,000 gave the most nearly typical results. A concentration of 1 in 100,000 was generally ineffective or caused only a temporary increase in amplitude in very vigorous preparations, but sometimes such a dose gave a typical but less marked response. Sometimes 1 in 25,000 caused only a progressive decrease in amplitude and rate without recovery. In one very vigorous preparation, however, 1 in 25,000 caused only an increase in amplitude.

Chloretone in the amount present in adrenalin chloride solutions of 1 in 25,000 to 1 in 50,000 (chloretone 1 in 5,000 to 1 in 10,000) was usually ineffective. In some cases, however, a dose of chloretone equivalent to that in 1 in 25,000 adrenalin chloride (chloretone 1 in 5,000) caused a moderate progressive slowing of the rate with little or no effect on the amplitude. With larger doses there was a marked decrease in both rate and amplitude without recovery.

DISCUSSION. It is apparent that the results reported above on the effect of adrenalin chloride on a sinus-auricle preparation confirm, in general, those reported by Macdonald (1925) on the effect of the "soloid" product and of epinine on the whole perfused heart. Inhibition of rate with recovery is typical, and frequently a decrease in amplitude followed by an increase occurs. The amplitude component is not constant, however. Concentrations of adrenalin chloride lower than 1 in 100,000 were never effective, which is at variance with the very weak effective concentration (1 in 5,000,000) reported by Macdonald (1925) for the "soloid" product. Possibly the chloretone in the adrenalin chloride reduced the apparent sensitiveness of the preparation.

Since both inhibition of rate and decreased amplitude of auricular beat (Bayliss and Starling, 1892) are typical vagus effects, the writer is inclined to interpret the effect of adrenalin on the elasmobranch heart to mean that adrenalin acts, in this case, on the parasympathetic endings, especially since Bottazzi (1902), Müller and Liljestrand (1918), and the writer (1930a) have been unable to demonstrate the presence of accelerator fibers by direct physiological means.

The presence of a well developed chromaphil system in elasmobranchs is difficult to account for on the basis of the recognized functions of adrenalin in the higher vertebrates. Reinforcement of the sympathetic system and temperature control seem especially not to be involved. Inasmuch as adrenalin has a vago-mimetic effect on the heart of these fishes, one may infer that this hormone does not take the place of a poorly developed

sympathetic system. A sort of inhibitory emergency function of the chromaphil system, however, may be correlated with what might be considered to be the usual reflex emergency response, namely, reflex inhibition of the heart and sometimes of respiration, caused by any unusual sensory stimulation at the gills, skin, or viscera. Schoenlein and Willem (1894) first pointed out the ease with which cardiac inhibition could be obtained from the pharyngeal cavity of the elasmobranchs, Scyllium and Torpedo. Lyon (1926) working on sand sharks (Carcharias) noted that a great variety of stimuli applied externally or to certain viscera caused inhibition of the heart, and Lutz (1930b) found that stimulation of the lateral line nerve, pericardial wall, ventricle of the heart, and certain abdominal viscera of Scyllium bring about reflex inhibition of the heart through the vagus. Emergency inhibition would serve to decrease absorption of toxic substances by the gills or, in case of injury especially to the gill vessels so directly connected with the heart, to prevent excessive bleeding from blood vessels probable lacking a vaso-constrictor innervation. Since inactivity is one type of primitive protective reaction, the inhibitory response to adrenalin may be an example of this reaction in an emergency apparatus which in higher vertebrates with an elaborate sympathetic system has more aggressive characteristics.

SUMMARY

1. Adrenalin chloride, 1 in 50,000 to 1 in 25,000 produced slowing or temporary complete inhibition of the beat of an isolated sinus-auricle preparation made from the heart of the elasmobranch fish.

2. The amplitude of beat may also decrease but both rate and amplitude recover, the latter frequently becoming temporarily greater than the original.

3. Chloretone in quantities equivalent to the amount present in adrenalin chloride of the concentrations used to produce typical effects was ineffective.

4. The inhibitory effect of adrenalin on the elasmobranch auricle is interpreted as the response of an unbalanced parasympathetic mechanism in a heart which lacks a sympathetic accelerator innervation.

5. An inhibitory emergency theory is suggested.

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THE EFFECT OF EXERCISE ON VENTRICULAR MINUTE-OUTPUT TIME

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Many attempts have been made in the past to measure and record the effects of exercise on the work of the heart. Erlanger's formula, $PR \times R$ has been both attacked and defended. Many efforts have also been made to determine the minute-output of the heart, all of which involve delicate and complicated procedures which lessen its practical application.

It occurred to us that an approximate indication of the work of the heart could be obtained by determining the length of the active systole, i.e., the ejection phase, as it is influenced by exercise, and in order to make determinations which would be comparable in all cases, it was decided to record the minute output-time: the number of seconds per minute during which blood was being ejected from the heart. This factor was obtained by multiplying the length of successive systoles in seconds by the actual rate for each cycle, the formula being:

$$M = SR \text{ or } M = \frac{60\,S}{C}$$

in which M = minute output time, R = rate, S = systole, and C = cycle length, all measurements being in seconds.

The exercise was performed on a stationary bicycle, the subject sitting as quietly as possible, the severity of the exercise being governed by a friction-brake applied to the rear axle of the bicycle, a large fly-wheel being substituted for the original rear wheel. The carotid pulse was recorded on the drum of a long-paper kymograph which revolved rapidly enough to make the vibrations of a 100 v.d. tuning-fork easily read.

The subjects were normal healthy men and women students between the ages of 21 and 36 years. Records were taken with the subject sitting at rest on the bicycle, during the beginning of the exercise, while the exercise was at its height, at the actual cessation of the work, and at regular intervals for at least 60 cycles after cessation of the exercise, each record embracing at least 15 successive cycles.

No attempt was made to estimate the actual amount of work done, the

object of the experiment being to determine the relative changes in the length of systole under the conditions of the experiment. However, two types of experiments were made, one with a very light amount of friction on the wheel: "light exercise," and a second determination with enough friction to make the exercise decidedly strenuous for the subject—"heavy exercise."

The records were read in such a way as to give an accurate statement of the length of successive systoles and cycles during the entire duration of the experiment. These results were then averaged in such a manner as to give composite curves of 15 successive cycles before and immediately after the beginning and the end of each variety of exercise. From the data thus

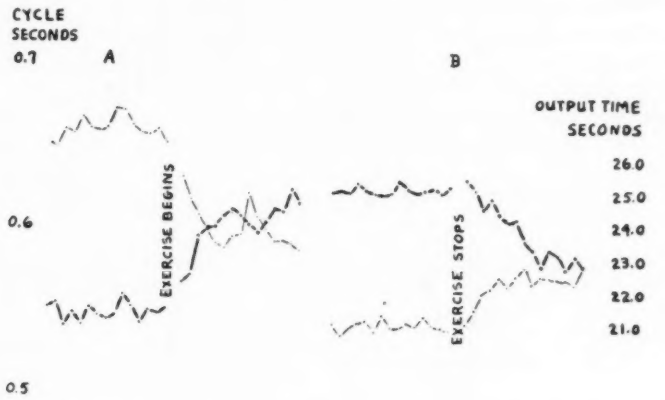

Fig. 1. Effect of light exercise on minute-output time. Heavy lines, minute-output time; light lines, cycle length.

obtained the appended figures for cycle length and minute-output time were obtained.

It is granted that this factor takes into consideration no changes in the rate of filling of the ventricle or of cardiac tonus, but is a function wholly of the relative time required to empty the heart.

Figure 1 shows the relationship of the length of cycle to the minute-output time in 15 successive cycles at rest, followed by 15 cycles of light exercise. Then there are shown the similar relationships in the 15 cycles immediately preceding and those immediately following the cessation of the exercise.

Figure 2 shows the corresponding relationships before, during, and after heavy exercise.

In each case the figures were obtained from records taken from eight different individuals.

An inspection of both figures shows that with the beginning of exercise the systole shortens much less rapidly than the cycle, so that it takes from three cycle-lengths in light exercise to ten or more in the heavy exercise for the adjustment to take place whereby the relationship between cycle and systole approaches constancy. In all cases the ejection period is relatively so much longer that the actual time during which blood is leaving the heart is greatly increased during the whole period of exercise.

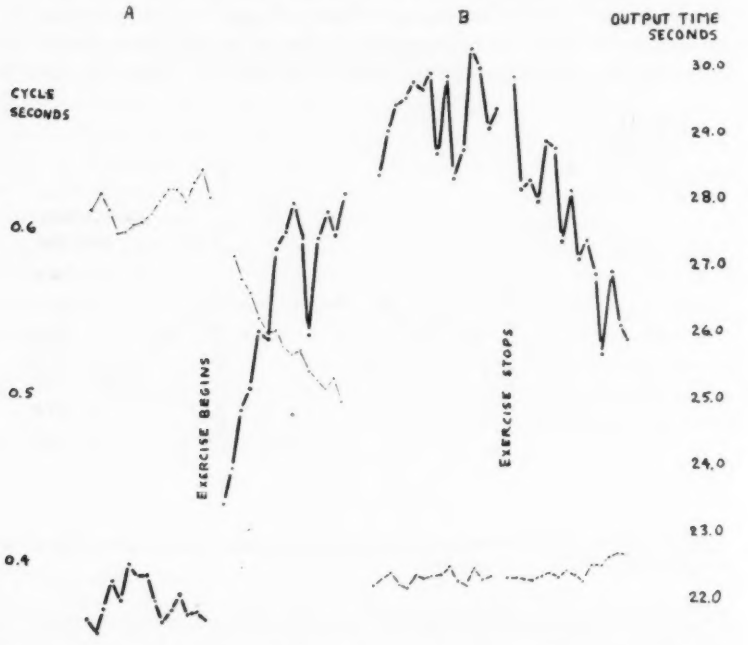

Fig. 2. Effect of heavy exercise on minute-output time. Heavy lines, minute-output time; light lines, cycle length.

This increase in minute-output time as compared with the actual cycle length of course is much greater the more strenuous the exercise, as shown by a comparison of figures 1 and 2.

In the case of light exercise, the cessation of the work is accompanied by reverse changes in the relative cycle and systole lengths, so that within a very few cycles after work stops the normal relationships are approached. After heavy exercise, on the other hand, cessation of work is immediately followed by a progressive decrease of the minute-output time even though the heart continues to beat as rapidly as while exercise was in progress.

These figures show that the human heart adjusts itself to the increased load it is called upon to carry as the result of exercise, aside from changes in tonus or rate of ejection, not only by an increase in rate, but also by both a relative and an actual increase in the time during which it is actually ejecting blood. This adjustment is such that the more work the heart has to do the greater will be the time during which it is furnishing blood to the tissues. Moreover, at the close of the work the ejection phase decreases even more rapidly than does the rate diminish, so that a return to normal conditions is greatly facilitated.

SUMMARY

By means of carotid pulse tracings from eight individuals seated on a stationary bicycle, the effects of light and heavy exercise on the cycle length and minute output-time is recorded, It is shown that the heart adjusts itself to increased demands not only by increased rate but also by relative increase in its ejection phase, and that this adjustment takes place more rapidly and therefore more efficiently than the heart-rate changes when the work ceases.

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THE BLOOD FIBRINOGEN LEVEL IN HEPATECTOMIZED DOGS AND AN OUTLINE OF A METHOD FOR THE QUANTITATIVE DETERMINATION OF FIBRINOGEN

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For the past several years we have been engaged in a study of the blood fibrinogen level in hepatectomized dogs. From the inception of this work we have been steadily more impressed, both with the complexity of the process of blood coagulation and with the difficulty in analyzing disturbances in the process of clotting. We shall give these matters particular attention.

Following removal of the liver we note a drop in blood fibrinogen, but the fall may not begin at once. The inception, and the rate of fall as well are variable, but all dogs which survive more than 10 to 12 hours show a very definite change. We are unable to confirm the work of Rosenthal, Licht and Melchior (1) to the effect that the fall takes place precipitously at about the 10th hour. We have noted periods when the change was rather rapid, but nothing which resembled the abrupt and outspoken drop indicated in their experiments. Our results are more in agreement with those of Drury and McMaster (2) who recently reported similar experiments performed on rabbits. In an earlier contribution Williamson, Heck and Mann (3) found fibrinogen changes inconstant following removal of the liver. More recently Mann, Bollman and Markowitz (4) have made a preliminary report expressing their belief that the presence of the liver is essential to maintenance of the fibrinogen level.

Since abundant evidence shows that fibrinogen regeneration normally is rapid (5, 6, 7, 8, 2), it follows that any conspicuous fall in level of blood fibrinogen is due either to a defect in the process of fibrinogen formation or to abnormal utilization or destruction of this protein.

The possibility of fibrinogen destruction by ferment action has been suggested by certain investigators, notably Nolf (9). On excluding the liver from circulation he ordinarily found no change in coagulability whatever, probably because the animals lived only a very few hours. If, however, the animals had been on a rich meat diet for several days before the operation, he occasionally found that, though the clot formed promptly when blood was removed, on standing the clot redissolved. He could

obtain the same result in hepatectomized dogs by the intravenous injection of peptone and this result could be obtained irrespective of diet. In certain experiments this destructive action, which in the early stages of the experiment affected the fibrin of the fully formed clot, in the later stages, rendered the blood completely incoagulable, probably due to action on the fibrinogen itself. We find evidence of similar changes when peptone is injected into dogs from which the liver is removed, but without peptone injection we do not observe these striking autolytic changes. We believe that the liver removal produces an interruption in the process of fibrinogen formation and that this is the essential factor in the fall of blood fibrinogen following simple removal of the liver.

THE QUANTITATIVE DETERMINATION OF BLOOD FIBRIN. Essentially there are two groups of methods for the quantitative estimation of fibrin. One of these involves the precipitation and collection of the fibrinogen by "salting out" or by isolation by other artificial physical or chemical means. In the other group of methods the blood or plasma is allowed to clot and the amount of fibrin formed is ascertained. This latter method has the advantage of being a measurement of a physiological reaction. The salt precipitation methods are distinctly more artificial, and the results they yield are also subject to difficulty in interpretation. We believe that no one now advocates the use of the earlier quantitative method involving measurement of the coagulum obtained by heating plasma to 58 to 60°C.

Many of the older workers attempted to remove the fibrin from the blood by whipping, but it was difficult to remove the blood cells completely without losing part of the fibrin. Furthermore, rather large amounts of blood were required for analysis. Cullen and Van Slyke (10) avoided certain of the difficulties by making their measurements on oxalated plasma instead of on whole blood. Measured amounts of plasma were recalcified and allowed to clot. The fibrin clot thus obtained was separated out and measured by the macro-Kjeldahl method. Foster and Whipple (11) obtained the coagulum in a similar way, but they weighed the clot instead of using the Kjeldahl technic. They were thus enabled to work with small amounts of plasma. Our own experience with the gravimetric method has been unsatisfactory when the fibrinogen content of the blood is low. We have employed a micro-Kjeldahl method for measurement of the clot. In this way we avoided using the large amounts of plasma required by the method of Cullen and Van Slyke. The advantages of this technic are particularly obvious when the fibrinogen content of the blood is below normal levels. While our work was still in progress another micro method was proposed by Wu and Ling (12). In their method the fibrin clot was digested and the tyrosin present in the digest was determined by means of a color reaction. Like our micro-Kjeldahl method, this offers a method for the analysis of small amounts of fibrin.

In devising our method, we have given particular attention to obtaining a complete clot, as free as possible from impurities. Cullen and Van Slyke (10) and Foster and Whipple (11) have used 20 to 30 cc. saline to each cubic centimeter of plasma. At such dilution the fibrin is readily sepa-

rated out from the serum and from the proteins so abundantly present in the latter. These workers also studied the influence of such factors as temperature, amount of calcium to be added and the question of time factors. Room temperature is satisfactory for these experiments. We have corroborated their work and in addition we have studied the effect of hydrogen ion concentration. In acid solution (pH 5.0–6.0) the amount of coagulum formed may be very great, possibly from inclusion of extraneous proteins along with the fibrin. In alkaline solutions the coagulum becomes progressively less, until at pH 9.3 to 9.5 no clot whatever is formed. That there is no permanent change in the clotting factors is shown by the fact that a normal clot forms when neutrality is restored. Nor is the failure to clot in alkaline solution due only to binding of calcium ion by hydroxyl ion. The addition of purified thrombin will not cause clotting as long as the alkalinity is maintained, but restoration of neutrality results in the formation of a clot. In making quantitative determinations it is not necessary to buffer the mixture. Measurement of pH shows that the plasma-saline mixture is approximately neutral, providing the reagents used are free of traces of acid and alkali.

The direct addition of pure thrombin to oxalated plasma would be the ideal method for obtaining clots for quantitative analysis. However, the preparation of pure thrombin has not been accomplished. We have prepared a product of thrombin from pig fibrin according to the technic of Howell (13) and have found that it was very effective in bringing about coagulation. We agree with Howell that with careful purification the product will contain relatively little protein coagulable by dilute acid and heat, but the solution does contain large amounts of nitrogen. Specimens which cause prompt coagulation of 20 to 30 times their volume of plasma often contain as much as 0.2 per cent nitrogen, and, under certain conditions, much of this nitrogen is retained in the final clot. This causes a very appreciable error in the quantitative estimation of fibrin. We have found that the inclusion of this extraneous nitrogen is minimal if the thrombin-plasma mixture has a pH of 7.0 to 7.4, but it is maximal in more acid solutions. It is of interest that this foreign coagulum can be formed even in the absence of fibrinogen, for, if the thrombin solution be added to serum, a cloud will form if the pH be reduced to 6.0, though neither substance alone becomes turbid at this acidity. Evidently at proper acidity substances in the thrombin solution react with substances in serum to form this cloud. We do not know whether thrombin itself is one of the reagents or whether it is some impurity which reacts with the unknown constituents of the serum. The problem is evidently one of some complexity, and, in the present state of our knowledge, we hesitate to recommend the use of "purified" thrombin in making quantitative estimation of fibrin.

The addition of serum to promote clotting as recommended by some workers is even more objectionable. Such serum also contains large amounts of nitrogenous impurities. Furthermore one cannot rely on the thrombin of such serum to clot plasma to which it is added, for the thrombin of serum becomes rapidly transformed into the inert metathrombin, and within a few minutes the coagulating power of serum may be almost nil.

In view of the objections to these coagulants we have adopted the use of cephalin to promote clotting. Cephalin supplies a substance which is decidedly necessary for clotting, and which may not be abundantly present in oxalated plasma, especially if the blood has been so thoroughly centrifuged that the platelets are removed. Under these conditions the addition of calcium permits only part of the prothrombin to be converted into thrombin. The maximal yield of thrombin can be obtained only by the artificial addition of thromboplastin (cephalin). We have made such an addition of cephalin a part of our routine in fibrin determination. According to Howell, this cephalin is needed to neutralize antithrombin; according to Morawitz, to unite with the prothrombin to form thrombin. In either case it is readily seen that the addition of cephalin produces promptly a maximal yield of thrombin, and this is important in view of the tendency of thrombin to degenerate into the inactive metathrombin.

Foster and Whipple (11) expressed the fluid from the clot by mechanical pressure exerted by means of a glass stirring rod. We find the method advocated by Wu and Ling (12) much more satisfactory when the clots are tough. They use very small glass rods and find that the fibrin can be isolated on these rods by a process of rotation, the serum being expressed as the fibrin strands are wound about the rod. We pour the clot on a large watch glass preparatory to this process of separation of the fibrin. If the rod is not more than 1.0 mm. in diameter the toughest clots can be handled by this technic. The bundle of fibrin thus isolated is rinsed with saline preparatory to further analysis. When the clots are too flabby or too stringy to be collected on a glass rod, we recover the fibrin by centrifugalization. After pouring off the fluid the fibrin is carefully washed with saline and separated again by centrifugalization.

Various methods have been suggested for measurement of this coagulum. Cullen and Van Slyke used the ordinary macro-Kjeldahl method with titration of the distillate. This method is quite precise if large amounts of plasma are available for analysis, but in case the fibrinogen content of the plasma is very low the amount of plasma used must be very large indeed, and excessive bleeding of the animal is required, especially if one wishes to run analyses in duplicate. The method of Foster and Whipple of weighing the dried clot before and after ashing seemed adequate until very small amounts of fibrin were encountered. Under such conditions there developed considerable experimental error, as judged by micro-Kjeldahl

controls and by failure to obtain good checks on samples run in duplicate or triplicate.

It is often necessary to analyze plasma with low fibrinogen content and it is desirable that we have a method which requires only small amounts of plasma. After experimentation we finally adopted a modified micro-Kjeldahl method. Attempts to read the results by direct Nesslerization of the digested clots proved unsatisfactory in many cases, due largely to excessive "clouding," so that it was necessary to distill the mixture and to Nesslerize the distillate. The increased work of this more complex method was rewarded by much more accurate and satisfactory readings. A miniature glass distilling apparatus was made and all samples were analyzed in this manner.

METHOD. Freshly drawn blood is promptly placed in a 15 cc. graduated hematocrit tube containing 2 cc. of 1.6 per cent sodium oxalate. After thorough mixing, the tube is centrifuged 30 minutes at 2500 revolutions a minute. From the graduations on the side of the tube the total volume of plasma in the tube can be determined, making it possible subsequently to correct for the dilution caused by the 2 cc. of oxalate solution which had been added to prevent clotting.

Ordinarily, 1 cc. of plasma is placed in a large test tube which contains 27 cc. of 0.9 per cent sodium chloride, 1 cc. of 2.5 per cent calcium chloride and 1 cc. of 0.4 per cent cephalin solution prepared according to the method of Howell (14). The mixture is allowed to stand for an hour or longer to insure complete coagulation, at the end of which time the clot is separated from the fluid in the manner described above. The clot thus obtained is rinsed in normal saline and is treated briefly with acetone and ether to remove the excess of cephalin. For this purpose the clot is first treated 15 minutes each with two changes of acetone. The acetone is then drained off and the clot thus freed of water is treated 30 minutes with ether, to remove the excess of cephalin. The ether is drained off and the fumes are removed by a current of air directed into the tube. The clot is then digested over a free flame with 1 cc. of the acid digestion mixture recommended by Folin and Denis (15). This latter part of the technic is identical with the micro-Kjeldahl technic in common use in many laboratories.

It is rarely possible to carry the digestion to completion without producing marked turbidity which interferes with the ordinary process of Nesslerization. To get rid of the turbidity and of the salts which may be present in excess, other workers (16) have advised distillation of such turbid digests, and in principle our technic is not different from that which they recommend. The digest is diluted with water and is carefully transferred to a 100 cc. Kjeldahl flask. Recently it has been difficult to procure flasks of this size, but suitable substitutes can be prepared by any glass blower by removing the side tube of ordinary long-neck Pyrex distillation flasks. The flask with the digest is then filled to about two-thirds capacity by the addition of distilled water, and a small piece of granulated zinc is added to provide a small stream of hydrogen bubbles. A small piece of paraffin about 0.5 cm. in diameter is added to prevent excessive foaming. Before connecting the flask to the condenser, 10 cc. of 40 per cent sodium hydroxide are added. The condenser is a small specially constructed water-jacketed glass condenser about 40 cm. in length. The inner tube is thin-walled and has an outside diameter of 10 mm. The outer tube has an inflow and outflow for water and is made of moderately thick-walled glass tubing having

an outside diameter of 14 mm. The two parts of the condenser are connected by two pieces of rubber tubing. As in the ordinary macro-Kjeldahl still, a cylindrical connecting bulb is placed above the distillation flask. In our apparatus it is specially constructed. The bulb is 2.5 cm. in diameter and 9.5 cm. long.

The distillate is collected as in ordinary Kjeldahl determinations by passing the end of the condenser tube to the bottom of a bottle containing, in this case, 1 cc. of the acid digestion mixture and a few cubic centimeters of water to cover the end of the condenser tube. About 70 to 80 cc. of fluid from the Kjeldahl flask are boiled over. The distillate is made up to a total volume of 100 cc., and to 10 cc. of this are added 5 cc. of Nessler's reagent. Standard solutions of suitable strength are prepared from ammonium sulphate, and these are treated with Nessler's reagent and the mixture is used for colorimetric comparison with the unknown.

We have made it a practice to run complete determinations in duplicate, and as a rule a third tube is run through as far as the process of digestion. This specimen is kept in reserve, to be used in case the duplicate samples are not in agreement. Very rarely do the duplicates differ more than a few per cent, and most of this discrepancy may be attributed to error in making the readings on the colorimeter.

When it is thought that the fibrinogen content of the plasma is low, it is well to use larger amounts of plasma, but rarely are more than 2 or 3 cc. required for each tube.

To conserve time we ordinarily connect a number of the condensers in series and distill a number of samples at the same time, as in macro-Kjeldahl determinations.

The amount of fibrin-nitrogen obtained from each cubic centimeter of the original plasma is multiplied by 6.25 to obtain the amount of fibrin-protein. The figures given by us are expressed in milligrams of fibrin per 100 cc. plasma.

Discussion. With our method we find that the plasma of dogs usually contains between 250 and 350 mgm. fibrin per 100 cc. These values are distinctly lower than those appearing in the older literature. After removing the clot, we have tested the remaining fluid with thrombin for the presence of unconverted fibrinogen. Our failure to obtain any evidence of clotting proved that the initial clotting was complete. We believe that the high values of older workers were due as much to impurities in the clot as to imperfect methods of analyzing the clot.

We call attention to the unreliability of certain commercial preparations of cephalin, and we advise that this substance be prepared according to the principles contained in an article by Howell (14). The cephalin thus prepared is divided into small portions which are placed in hermetically sealed tubes containing nitrogen gas. These are kept in the ice box. Preserved in this way, the cephalin retains its thromboplastic character for many months.

In making fibrin determinations one must bear in mind the fact that coagulation may not be complete if there is a deficit of thrombin or if anti-coagulating substances are present in unusual amounts. When this is suspected it is well to add purified thrombin to the fluid from which the clot had been taken for analysis. If no clot forms under these conditions one may feel certain that the maximal yield of fibrin had been obtained.

Finally, the analyst must keep constantly in mind the fact that the clots of certain abnormal types of blood tend to dissolve spontaneously, sometimes in relatively short space of time. Suitable controls should be set up to settle this point.

The precautions and controls we have suggested are mentioned to emphasize the need for care on the part of those making quantitative studies on fibrin. Special controls are demanded in special cases, and familiarity with the principles of coagulation should serve as a guide in planning such controls. The making of such analyses cannot be left to rule of thumb.

BLOOD FIBRIN IN HEPATECTOMIZED DOGS. *Method of removing liver.* Removal of the liver was effected according to a technic similar in principle to that described by Mann and Magath (17). We have modified the technic in certain ways. The tying of the portal vein at the second stage operation results in death in a very high percentage of cases. We have made it a practice to occlude the portal vein at two stages, and for this purpose we adopted the use of metal bands as used by Halsted (18) for the occlusion of vessels. We made use of lead bands in our earlier experiments, but finding them less permanent than aluminum, we later resorted to the use of this latter metal. Toward the end of the first stage operation (reverse Eck fistula) the band was placed in position about the portal vein, to be tightened at subsequent operations. In those dogs whose condition seemed satisfactory at this time the band was compressed approximately one-third just before the abdomen was closed. Unless the band is compressed somewhat at this time, it is necessary to effect complete occlusion at two subsequent operations. Mann and Magath had recognized the need of gradual occlusion of the portal, and in selected cases they transfixed the portal vein with a silk suture and ligated only half of the vein. Complete occlusion was effected at the third operation. We find the occlusion with metal bands advantageous, for fewer adhesions form and very little exposure is needed to locate and compress the band.

We found it unnecessary to use positive pressure anesthesia, as used by Mann and Magath. In our experience the excessive motility of the diaphragm due to the removal of the liver caused no respiratory embarrassment, either during or following the operation. In all of our experiments we used the ordinary inhalation anesthesia.

The fibrinogen level in the blood stream represents a balance between the process of formation and that of utilization or destruction. This balance can be disturbed by replacing the normal circulating blood with defibrinated blood, and this may be done by repeated bleeding with reinjection of the blood after defibrination, or by massive bleeding with simultaneous injection of an equal quantity of defibrinated blood from other animals of the same species.

Goodpasture (6) reported in 1914 that after "complete defibrination" enough fibrinogen is regenerated in 15 minutes to form a large clot, and in 30 minutes a sample of blood will clot firmly enough to permit inversion of the tube. In general this conclusion is in agreement with earlier work (5). Very clearly this indicates regeneration, or more probably still, the outpouring of fibrinogen held in storage; still we cannot look upon these observations as being quantitative. We now know that the rigidity of the clot is a very poor index of the amount of fibrin present. We

can show that the character of the clot can be influenced by varying the conditions under which the clot is allowed to form. Addition of cephalin, or better still, of purified thrombin, to recalcified plasma increases the rigidity of the clot to a striking degree without necessarily increasing the amount of fibrin demonstrable by Kjeldahl determinations.

The work of Meek (7) also indicates regeneration within the first few hours. By perfusion he reduced the fibrinogen from about 157 mgm. per 100 cc. to about 17 mgm., and within 4 hours he found that the level had risen to about 35 mgm. The regeneration during the first 4 hours is spoken of by Meek as "100 per cent increase," though obviously it is so only in relation to the very low values prevailing immediately after perfusion. It would seem that this phrase has been taken by some to imply complete regeneration during the first 4 hours, though it is clear that this was not Meek's interpretation. In reality the rise of about 18 mgm. represents only 13 per cent restoration of the fibrinogen removed. At this rate, one can well believe that 24 hours would elapse before regeneration was complete, though Meek does not seem to have followed the animals throughout this entire period of recovery. This longer

TABLE 1

Dog 26-125. Regeneration of fibrinogen by normal dog

TIME	HEMATOCRIT (CELLS)	FIBRINOGEN PER 100 cc. PLASMA	RELATIVE PER CENT FIBRINOGEN
	per cent	*mgm.*	
Before perfusion	53.3	230	100
1½ minutes after	59.1	90	39
15 minutes after	57.6	89	39
1 hour after	54.3	80	35
6 hours after	52.3	102	44
24 hours after	47.5	285	124

period of regeneration is quite in accord with our own experience on regeneration following such perfusion. Foster and Whipple (8), also working on the dog, found definite evidence of regeneration in the first few hours, but regeneration was by no means complete until 24 hours had elapsed.

Dog 26–125. Regeneration of fibrinogen by normal dog (see table 1). Mongrel, 8.1 kgm. Ether anesthesia. Bled 1300 cc. from artery. Simultaneously, 1300 cc. fresh warm defibrinated blood from other dogs injected into vein. Inflow rate kept approximately equal to outflow rate. The perfusion took 3.5 minutes. Dog somewhat depressed for 2 hours, but in good condition thereafter. Fibrinogen values shown in table 1.

Dog 26–129. Regeneration of fibrinogen by normal dog (see table 2). Poodle, 5.5 kgm. Technic of perfusion as in case of dog 26–125. Total exchange of 1050 cc. in 3.5 minutes. Prompt recovery. Fibrinogen values shown in table 2.

The results of two of our perfusion experiments are shown in tables 1 and 2. The fibrinogen falls from a normal level of about 230 mgm. per 100 cc. to about 90 mgm. There is slight, though definite, regeneration

during the first 5 or 6 hours, and recovery is practically complete at the end of 24 to 48 hours. The maximal values reached within 24 to 48 hours may be distinctly above the normal.

Evidently regeneration is much more rapid in rabbits, judging by the recent experiments of Drury and McMaster (2). They find complete restoration of normal values within 5 hours following perfusion.

The source of fibrinogen in the body has been the subject of much conjecture and of no little experimentation. The earlier literature is reviewed by Meek (7). Most of the work has gone to show that the liver is vitally concerned in the maintenance of the normal fibrinogen level in the blood stream. Other organs, including intestine, spleen and bone marrow have been brought into the discussion. Chloroform administration causes marked necrosis of liver tissue, and in dogs thus poisoned there soon develops a marked deficiency in the fibrinogen of the blood. Whipple and

TABLE 2

Dog 26-129. Regeneration of fibrinogen by normal dog

TIME	HEMATOCRIT (CELLS)	FIBRINOGEN PER 100 CC. PLASMA	RELATIVE PER CENT FIBRINOGEN
	per cent	*mgm.*	
Before perfusion	47.0	250	100
2 minutes after	45.2	86	34
15 minutes after	41.0	74	30
1 hour after	41.5	80	32
6 hours after	43.0	97	39
28 hours after	38.6	127	51
50 hours after	39.0	235	94

Hurwitz (19) have given this matter careful study and have correlated the fibrinogen deficit with the amount of liver injury. They cite older literature which contains isolated references to fibrinogen deficit following poisoning by chloroform or by phosphorus. More recently Foster and Whipple (20) have shown that the fibrinogen level can be affected in many ways. An increase in the fibrinogen level results from small doses of chloroform or from tissue destruction, whether mechanical, chemical or bacterial in origin. It would seem that such a rise in blood fibrinogen level is due to stimulation of formation or to mobilization of this protein. They also show that under other circumstances (large doses of chloroform or phosphorus) the fibrinogen level decreases, indicating they believe, a diminution in formation due to marked liver injury.

A number of investigators have attempted to remove the liver, or to exclude it effectively from circulation. Ligation of the hepatic artery and portal vein in Eck fistula dogs interrupts the circulation to the liver almost completely, and according to Meek, such dogs cannot reform fibrino-

gen removed by perfusion. The earlier workers on liver removal were greatly hampered by difficulty in keeping the animals alive for more than a very few hours at most. The improvements introduced by Mann and Magath (17) consist partly in improved operative technic, but no less important was their discovery that the life of the animal could be greatly prolonged by the administration of sugar. With these improvements it is possible to keep the animal in good clinical condition for 20 hours or more in favorable cases. In such animals one may well expect alterations in the factors of coagulation not to be observed in animals which survive 5 hours or less. Aside from a few brief notes by Williamson, Heck and Mann (3), it appears that Rosenthal, Licht and Melchior (1) were the first to present data on fibrinogen level in dogs deprived of liver by the newer technic. They report finding relatively little alteration for the first few hours, but at about the 10th to 12th hour they noted a very abrupt precipitous fall in the fibrinogen level. After this period the blood was said to be almost completely incoagulable from lack of fibrinogen. The very abruptness with which this fall occurred suggests that fibrinogen had been destroyed, possibly by ferment action. Unfortunately they do not seem to have given this point special study. Nothing is said concerning whether the clots which did form showed any tendency to dissolve in the course of time. It is possible that the clots dissolved partially during the process of analysis or that the fibrinogen itself was destroyed, perhaps by ferment action. In any case it is difficult to understand the sudden change in metabolism at about the 10th to 12th hour—a change resulting in the very rapid fall in fibrinogen. We have observed nothing comparable to this in our own experiments (see chart A). The rate of fall was not constant throughout the experiment and it varied in different animals, as we will show, but at no time was there any abrupt fall like that reported by Rosenthal, Licht and Melchior. We must confess that we have little confidence in the method by which these workers determined the fibrinogen content of the blood. They used the series of dilution method of Wohlgemuth (21). The plasma in varying degrees of dilution is caused to clot by adding fresh serum containing thrombin. The greatest dilution consistent with clotting is noted. The amount of fibrin present is assumed to be inversely proportional to this degree of dilution. Thus, if clotting occurs when the plasma is diluted 1 to 60, but not in more dilute mixtures, it is assumed that there are 60 "fibrin units;" if it clots at 1 to 100 dilutions, there are 100 units, etc. We believe that critical workers will discard this method in favor of one where the fibrin is actually recovered and estimated by weighing or by Kjeldahl or other chemical means. The coagulation of such dilute mixtures is a very uncertain matter, especially in the presence of large amounts of serum introduced to supply the fibrin ferment. We might even anticipate that these flimsy clots would redissolve if any fibrinlytic

ferments whatever were present in the mixture. It is to be noted that, like Wohlgemuth, these workers allowed the tubes to stand 24 hours before making the readings. It may well be that the abrupt fall noted by them at the 10th to 12th hour is associated in some way with their method of estimating the amount of fibrin.

Dog 27–82. Liver removal. Fibrinogen content of blood (see chart A). Mongrel, 19.2 kgm. Reverse Eck fistula and partial compression of portal vein, November 9, 1927. Complete occlusion on December 8. Liver removed at 8:10 a.m., November 22, 1928. Liver weighed 420 grams. Ten per cent glucose intravenously: 30 cc. at 9:32 a.m.; 75 cc. at 11:22; 120 at 1:15; 60 at 2:30; 85 at 4:15; 120 at 5:45; 120 at 7:15; 90 at 8:30; 100 at 10:00; 120 at 11:45; 120 at 1:30 a.m.; 90 at 3:00. Died at 4:20 a.m. Clinical condition good and at 17th hour dog still walked about occasionally, but thereafter rather dull. Some little muscular twitching shortly before death, but less than often seen.

At autopsy, connective tissues slightly yellow. About 100 cc. thin bloody fluid in peritoneal cavity. Did not clot on standing. Eck fistula widely patent. No congestion of intestines. Good development of collateral veins in subcutaneous tissues and body wall.

Coagulability of blood tested before operation and also at intervals later on. Blood drawn from jugular vein into well-vaselined syringe. Blood allowed to clot in clean glass tubes of about 8 mm. bore. All samples, including one taken just before death, clotted in 4 to 5 minutes. None of the clots autolyzed on standing 30 hours at room temperature.

In taking samples for fibrinogen determination we avoided as far as possible taking the specimens shortly after injection of the sugar solution, for this causes transient dilution of the blood. Despite these precautions, there is still some slight dilution effect as the following hematocrit figures show: Before liver removal blood contained 53.0 per cent cells by volume; 6 hours after liver removal, 49.0 per cent; 12 hours, 48.5; 16 hours, 54.2; 20 hours, 47.4 per cent. Fibrinogen values shown in chart A.

Dog 26–139. Liver removal. Fibrinogen content of blood (see chart A). Male setter, 23.5 kgm. Ether anesthesia, reverse Eck fistula April 27, 1927. Portal ligated August 3. Liver extirpation January 5, 1928 at 10:10 a.m. Ten per cent glucose: 12:45 p.m., 50 cc.; 1:30 p.m., 75; 3:50, 100; 5:00, 100; 7:00, 100; 8:35, 75; 10:35, 120; 12:10 a.m., 100 cc. Died at 1:10 a.m. Some muscular twitching during last 3 hours. Autopsy showed 100 cc. bloody fluid in peritoneal cavity. Eck fistula patent. Spleen large and dark red. Intestinal mucosa reddened, especially in duodenum.

Blood samples taken in oxalate, centrifuged. Specimen taken before liver removal contained 44.7 per cent cells; at 10:31 a.m., 44.7; 11:31, 45.0; 3:50 p.m., 47.5; 7:00 p.m., 44.0; 10:35, 43.6; 12:10, 40.2. Fibrin-values shown in chart A.

Dog 26–134. Liver removal. Fibrinogen content of blood (see chart A). Bull terrier, 16.0 kgm. October 25. Ether anesthesia, reverse Eck fistula. Metal band placed loosely about portal vein. Band partially compressed on November 17. Completely compressed on December 12. Liver extirpation June 20, 7:45 a.m. Ten per cent glucose: 11:45 a.m., 60 cc.; 1:45, 60 cc.; 3:30, 100; 5:15, 100; 6:55, 100. Death at 8:30 p.m. Convulsions noted during last few hours. Severe during last few minutes. Autopsy showed 200 cc. bloody fluid in peritoneal cavity. Eck fistula patent. Kidneys congested. Intestines very slightly so.

Hematocrit readings: Before operation 54.6 per cent cells; at 11:45 a.m., 56.2 per cent; 5:15, 51.0; 8:27, 53.3. Fibrin values in chart A.

Dog 26–37. Liver removal. Fibrinogen content of blood (see chart A). Mongrel, 16 kgm. Ether anesthesia, reverse Eck fistula, September 21, 1926. Portal ligated November 11. Dog grew and stored much fat and on January 17, 1928 weighed 27.8 kgm. Ether anesthesia. Liver extirpation at 10:20 a.m. Ten per cent glucose intravenously: 11:43 a.m., 50 cc.; 1:45 p.m., 50; 3:40, 75; 6:15, 75; 8:20, 100; 10:30, 150; 12:20 a.m., 50; 1:25, 75. Died at 2:35 a.m. Severe convulsions during last 4 hours. Autopsy negative.

Hematocrit values: 9:15 a.m., 53.5 per cent cells; 11:43, 59.0; 3:40 p.m., 58.8; 8:20, 59.0; 12:20 a.m., 61.0; 1:25, 59.0; 2:36, 63.5. Plasma fibrin values shown in chart A.

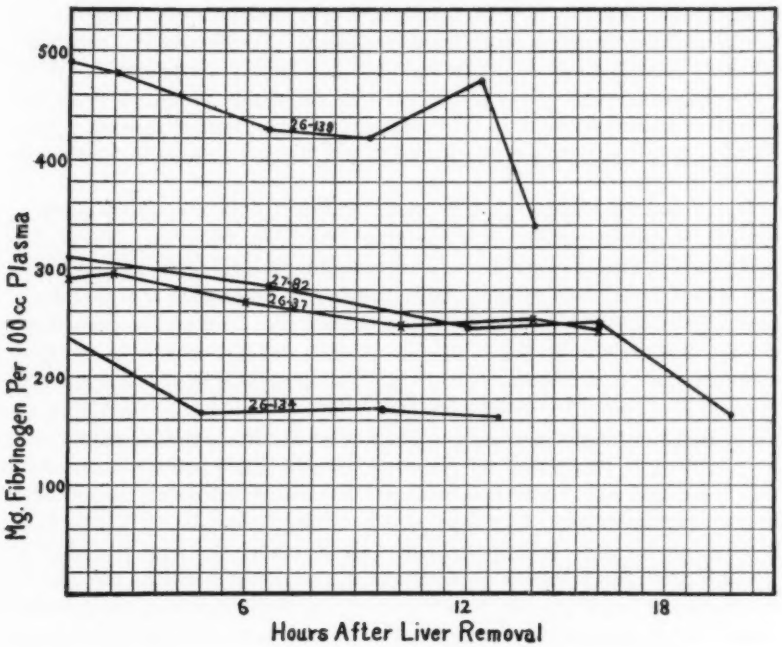

Chart A

Our fibrin studies following liver removal are summarized in chart A. The protocol of dog 27–82 is presented in some detail. Our other experiments are similar and the protocols are given in briefer form (dogs 26–139, 26–134 and 26–37). As chart A shows, the initial fibrinogen level varies in different animals, but, in our experience, all lie within the normal range. Evidently the previous exclusion of portal blood from the liver did not produce serious, or at least not prolonged, disturbance in the normal values. This is in accord with most of the older workers who observed no change in fibrinogen following ligation of the portal vein in Eck fistula dogs.

In most cases, one notes from the onset a gradual decrease in fibrinogen values beginning almost at once, and, at the end of 12–15 hours, there is a loss of somewhat over 20 per cent of the original fibrinogen present. In one experiment lasting 20 hours approximately half of the fibrinogen had disappeared. This represents a true loss of fibrinogen and not dilution of the blood. Obviously, the injection of sugar solution causes some dilution of the circulating plasma, and one can demonstrate this dilution by taking hematocrit readings at proper intervals. We recognized this source of error, and as far as possible our samples for analysis of fibrinogen were taken an hour or more after such injections. The hematocrit readings taken at such times showed that the fluid had largely disappeared. With these precautions the ratio of packed cells to plasma varied little in the course of the experiment, as the data given in the protocols show. Any changes noted are far too small to account for the change in fibrinogen shown in chart A.

Drury and McMaster (2) have noted that in rabbits, also, the fibrinogen level falls spontaneously after the liver has been removed. They also performed perfusion experiments on hepatectomized rabbits. The fibrinogen was thus reduced to low levels, and in the absence of the liver they found little or no evidence of fibrinogen regeneration. We have performed similar experiments on dogs, but our animals did not survive more than a few hours. A prominent feature in these experiments was the marked hemorrhage about the operative site. The tendency to bleed was so marked that pressure applied for 15 minutes at the site of venepuncture was ineffective in preventing the formation of large hematomata. We feel from our experience that many complicating factors are introduced in such perfusion experiments in hepatectomized animals. The injection of large amounts of defibrinated blood must disturb the ferment balance. We interpret the excessive hemorrhage in our experiments as one of the manifestations of what may be a widespread maladjustment in coagulating factors, and not alone a deficit in fibrinogen. Apparent failure in regeneration, as manifested by failure to recover fibrin for analysis, may well be explained on such a basis.

The fall in blood fibrinogen after hepatectomy adds another link to the chain of evidence which shows that the liver is vitally concerned in the maintenance of the normal levels of this protein. The ability of the body to reform fibrinogen removed by perfusion is very great indeed, as we have shown. We believe that the liver is essential to the formation of this substance. We know little about the normal utilization of fibrinogen, and less still about utilization under abnormal conditions. Foster and Whipple (20) stress the utilization in and about areas of injury. The fibrinous exudates of inflammation are known to all. Foster and Whipple suggest also that fibrinogen is retained in areas of coagulative necrosis and that it

contributes to the formation of the necrotic mass. They feel that in normal "wear and tear" fibrin is being used to close numerous tiny leaks in smaller vessels—leaks which result in part from innumerable small injuries, traumatic and otherwise. In our experiments some little fibrinogen must be utilized in and about the operative site, but in most cases this is not sufficient to cause any immediate fall in the blood fibrinogen level. Autopsies performed immediately after death show that there has been no widespread intravascular clotting to cause the fall in blood fibrinogen.

Dog 28–213. Liver removal. Peptone shock. Autolysis of blood clots. Pointer, 26.5 kgm. Mixed diet. Ether, reverse Eck fistula March 19, 1929. Metal band placed loosely about portal vein. Partial compression of band on May 13. Complete compression on June 27. Liver removal January 29, 1930, 10:10 a.m. Just before taking animal from table at 10:30, 60 cc. 10 per cent glucose intravenously. Prompt recovery. Ten grams Witte's peptone intravenously at 12:04 p.m. Dog became stuporous and died at 1:10 p.m. No sugar aside from the single injection above mentioned.

Blood withdrawn from jugular vein allowed to clot in small glass tubes. Blood taken before liver removal clotted in 5 minutes. Clot firm and rubbery. No autolysis on standing 48 hours at room temperature. Sample taken 5 minutes after injection of peptone clotted in 12 minutes. Clot not quite so firm as above. Sample 33 minutes after peptone clotted in 10 minutes; that taken 5 minutes before death clotted in 6 to 8 minutes. Last two clots flabby. All clots formed from blood drawn after injection of peptone autolyzed. The 5-minute sample had become flimsy in an hour. Completely dissolved in 3 hours. Autolysis in samples taken 33 minutes after peptone and 5 minutes before death even more rapid. Complete dissolution in 2 hours.

To another series of tubes small amounts of cephalin were added. Autolysis just as prompt as in the above series.

In a third series oxalated plasma was caused to clot by adding calcium. Clots from samples taken before operation quite firm. Rather flabby clot in sample taken 5 minutes before death. Others intermediate. No autolysis in 72 hours. In a fourth series the oxalated whole blood was allowed to stand 8 hours before being centrifuged to obtain the plasma. Results identical to third series where oxalated blood was centrifuged at once.

Quantitative fibrin tests on third and fourth series (milligram per 100 cc. plasma). Prompt centrifugalization: before operation, 276 mgm.; immediately before peptone, 204; 5 minutes after peptone, 197; 33 minutes after peptone 131; 5 minutes before death 126 mgm. Fourth series (8 hrs. delay in centrifugalization): before operation, 315; immediately before peptone 212; 5 minutes after peptone, 204; 33 minutes after peptone, 145; 5 minutes before death, 130. In all of these cases the recalcified plasma, mixed with saline, was allowed to stand 45 to 60 minutes to effect complete coagulation. The clots were then separated out and analyzed by the micro-Kjeldahl method already described.

Dog 27–77. Liver removal. Peptone shock. Autolysis of blood clots. Mongrel, 32 kgm. Ether anesthesia, reverse Eck fistula, metal band placed loosely about portal vein. Band compressed in two stages at monthly intervals. Liver removed 6 months later. Liver out at 11:20 a.m. Intravenous glucose (50 cc. 10 per cent) at 11:45 a.m. At 2:00 p.m. 75 cc. more given. Good clinical condition. At 2:20, 12

grams Witte's peptone intravenously. Dog went promptly into convulsions. Remained in coma until death 7 minutes later. Autopsy showed no hemorrhage. Fistula patent. Various organs normal.

White blood cell count before operation, 8600; 2 minutes before death, 3650. Platelet count by sedimentation technic, 116,000 per cubic millimeter before peptone and only 29,000 2 minutes before death.

In all cases whole blood clotted promptly in small test tubes (4 to 5 min.). Clots still well formed at end of 3 hours, but in 8 hours at room temperature clots had entirely redissolved in the specimens taken 2 minutes before death and at death. No autolysis whatever in clots of samples taken before peptone, nor did they dissolve in the following hours. Quantitative fibrin determinations not made, but firmness of clots after peptone injection suggests little or no reduction.

Regarding other ways in which fibrinogen can be utilized or destroyed, our knowledge is most incomplete. Conceivably, under abnormal conditions, the tissues may destroy fibrinogen in a most unusual and rapid manner, possibly by the liberation of ferments normally held in abeyance. There is very little proof of such a contention, but it is known that under experimental conditions, or in certain diseases of the liver, the clots which form may undergo rapid autolysis in standing. In a few minutes or a few hours a tough rubbery clot may disintegrate and the blood become perfectly fluid. The literature on this subject is most fragmentary and the nature of the phenomenon is poorly understood. Nolf (9) has done considerable work on this subject, but we must confess finding his statements often in apparent conflict, and in many places the data are regretably lacking. There is little evidence to show exactly how these clots are destroyed and still less to indicate whether the soluble fibrinogen of the plasma can be similarly destroyed prior to being converted into fibrin.

We submit two experiments which have a general bearing on this subject (dogs 27–77 and 28–213). In each case the liver had been removed and the dog was in good clinical condition. A solution of Witte's peptone was injected intravenously. This caused marked clinical disturbance. One animal died in convulsions 7 minutes later; the other became progressively weaker and died about an hour following the injection. In both cases, blood was removed 5 minutes or more after the injection of peptone, and samples of this blood were allowed to stand. In all samples clots formed within a few minutes, but on standing for several hours the clots underwent dissolution, and the blood became perfectly fluid. Control samples taken before the operation showed no autolysis within a period of 24 hours. In the case of dog 28–213, samples of blood were also taken in oxalate. The clear plasma obtained on centrifugalization clotted promptly on being recalcified, but unlike the clotted whole blood, the clots did not redissolve on standing. This might be taken to indicate that oxalate had destroyed ferments which were abnormally present but this seems rather unlikely. We are more inclined to believe that the

cellular elements of the blood must be present in the clot if autolysis is to occur. This observation is of interest from the standpoint of quantitative fibrin determinations, where oxalated plasma, freed of cells, is mixed with calcium and allowed to clot. The tubes are ordinarily allowed to stand 30 minutes or longer to ensure complete coagulation. It would be most disturbing if the clots which form from such oxalated plasma had the same tendency to autolyze which clotted whole blood sometimes shows. Our analytical procedure gains further support from the fact that oxalated plasma of liverless-peptone dogs may be allowed to stand many hours in contact with the blood cells before being centrifuged without affecting the amount of fibrin eventually recoverable from such plasma (see protocol of dog 28–213). It is evident that neither ferments of the blood nor the blood cells themselves destroy the fibrinogen until it has been converted into fibrin.

It is noted in the protocol of this peptone dog (no. 28–213) that the clots which form after peptone are flabby, and this is true even when cephalin is added to insure complete coagulation. It was, therefore, of interest to make quantitative determinations of the amount of fibrin present. The oxalated plasma was mixed with saline, calcium chloride and cephalin according to our usual technic and allowed to stand. The clots formed promptly. They were removed for analysis within 45 to 50 minutes. The figures given show that the fibrin falls steadily from a normal initial value of 276 mgm. per 100 cc. plasma to a final value of 126, shortly before death. It is noted that the fall is particularly rapid during the half-hour immediately following the injection of peptone. The total fall recorded represents a loss of half the circulating fibrinogen, and the fall took place in a period of two and one-half hours following removal of the liver. The change is brought on in some way by the peptone. We have never observed such a striking fall in uncomplicated hepatectomized dogs.

These two experiments show beyond doubt that under abnormal conditions clotted whole blood may undergo rapid autolytic changes. The rapid fall in blood fibrinogen following hepatectomy and intravenous peptone suggests that the fibrinogen of the circulating blood may be used up very rapidly. Admittedly, our data on this latter point are meager, and much more work is necessary.

This question of abnormal destruction of fibrinogen is of great interest in connection with any studies on blood fibrinogen level. We believe that the fall in fibrinogen following liver removal without peptone is largely an expression of deficient formation of this protein. We believe that all evidence points to the conclusion that the liver is essential to the formation of fibrinogen, yet this need not blind us to the fact that the rate of fibrinogen utilization may be quite variable, and at times may be very rapid. The evidence suggests that such rapid utilization may at times

be associated with the phenomenon of clot autolysis, as with peptone, though it is not quite clear just what relation can exist between the autolysis of fully-formed fibrin and the rapid utilization of the circulating fibrinogen. When peptone is not injected, the liverless dogs show disappearance of fibrinogen, but, as the protocol of dog 27–82 shows, samples of clotted blood showed no sign of fibrin autolysis. We are inclined to believe that in such uncomplicated cases of hepatectomy the fall in blood fibrinogen is a result of defective formation, combined with utilization which is probably little in excess of normal.

SUMMARY

Experiments are reported to show the effect of hepatectomy on the blood fibrinogen level in dogs. The liver removal is effected by a method similar to that of Mann and Magath, but certain modifications are introduced to reduce the mortality rate during the operations preliminary to removal of the liver.

Following removal of the liver there is a gradual fall in blood fibrinogen. Commonly 20 to 50 per cent of the fibrinogen disappears within 12 to 20 hours. Very clearly the normal balance between formation and utilization is disturbed.

All evidence available points to the conclusion that the liver is essential to the formation of fibrinogen. There is evidence to indicate that the utilization or destruction of fibrinogen in the body is quite variable and may be very rapid under certain abnormal conditions.

Certain technical matters relating to quantitative estimation of blood fibrin are discussed. The use of purified cephalin to promote clotting is proposed, and a modified micro-Kjeldahl method is recommended for analysis of the clot obtained. A discussion is given of the effect of hydrogen ion concentration on the yield of fibrin and upon the tendency of the clot to carry down protein and other materials contained in plasma. Certain other pitfalls in the determination of fibrin are pointed out, and precautions and control procedures are discussed.

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THE INFLUENCE OF THE PERICARDIUM ON ACUTE CARDIAC DILATATION PRODUCED BY VAGAL STIMULATION

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The controversy regarding the protective action of the pericardium was reviewed in a previous paper (Van Liere and Allen, 1927). It was shown in that paper that the pericardium tends to restrain acute cardiac dilatation in severe degrees of anoxemia, but that this degree of anoxemia is probably greater than any which occurs physiologically. In order to study still further any restraining influence which the pericardium might exert on the heart, vagal stimulation was used instead of anoxemia to produce acute cardiac dilatation.

PROCEDURE. The experiments were done on barbitalized dogs. A tracheal cannula was inserted and the vagus nerves exposed. No further operative procedures were carried out at this point. A control x-ray picture was taken of the dog's heart. The distance from the plate to the target of the tube was one meter. The length of exposure was 5 seconds. The vagus nerve was then stimulated with a sufficiently strong current to insure cardiac standstill, after which a second picture was taken.

Artificial respiration was administered to the animal and the pericardium was incised. This was done by making a small opening in the 4th or 5th interspace over the region of the cardiac apex. The pericardium was grasped with a small hemostat, slit longitudinally its entire length, but not entirely removed. As stated in a previous paper, considerable difficulty is experienced in taking x-ray pictures of the heart after removal of the pericardium, because it is apt to rotate. It is necessary that the animal be placed in an exact antero-posterior position. This rotation, however, can be practically abolished by incising the pericardium so that it cannot restrain the heart, but leaving enough to form a hammock in which the heart may lie. The wound was partially closed, the lungs were forcibly distended and held until after complete closure. Artificial respiration was then discontinued and the animal permitted to resume its normal breathing.

The animal was allowed two hours to recover from the effects of the operation. It was again placed under the x-ray tube and a post-operative control picture was taken. The vagus was stimulated while another exposure was made. Every effort was made to duplicate as closely as

possible the procedure carried out previous to pericardotomy. In several instances a number of x-ray pictures were taken to check the results. In the table, however, only the average figures are given. The cardiac silhouette was traced with a pen and the area measured by a planimeter.

RESULTS. The following table summarizes the results.

TABLE 1

DOG	WEIGHT	CARDIAC AREA—PERICARDIUM INTACT			CARDIAC AREA—PERICARDIUM CUT			INCREASE IN VAGAL RESPONSE AFTER PERICARDOTOMY
		Normal	Vagus stimulation	Increase in cardiac area during vagal stimulation	Normal	Vagus stimulation	Increase in cardiac area during vagal stimulation	
	kgm.	*sq. cm.*	*sq. cm.*	*per cent*	*sq. cm.*	*sq. cm.*	*per cent*	*per cent*
1	8	38.70	45.15	16.66	37.73	45.79	21.36	4.70
2	7.5	39.66	45.15	13.84	39.99	47.08	17.72	3.88
3	3	17.73	20.64	16.41	18.06	21.60	19.60	3.19
4	9.1	43.21	48.37	11.94	43.86	50.31	14.70	2.76
5	6.6	33.54	39.99	19.23	33.54	40.63	21.13	1.90
6	9.5	48.37	53.53	10.66	48.37	54.82	13.33	2.67
7	10.2	49.34	54.18	9.80	49.02	58.05	18.42	8.62
8	21	79.33	87.07	9.76	81.27	93.84	15.46	5.70
9	16.6	58.69	63.21	7.70	63.21	74.17	17.33	9.63
10	5.7	26.44	32.89	24.39	27.73	36.76	32.56	8.17

DISCUSSION. It will be noticed in the preceding table that the heart showed a greater response to vagal stimulation, as measured by the cardiac silhouette, after the pericardium had been incised. The largest increase was 9.63 per cent and the smallest 1.90 per cent.

The question naturally arises as to whether or not there was a complete cardiac standstill in each case. In several animals blood pressure tracings were taken. The x-ray pictures were made after the tracing showed a definite cardiac standstill and caution was observed that the heart did not escape from this inhibition until after the exposure. In these animals the exposures were made from 2 to 5 seconds after cardiac standstill. In other animals the femoral pulse was checked during the exposure. It may be said that it was very difficult and sometimes impossible to produce cardiac standstill in airedale dogs. Animals in which cardiac standstill could not be produced were not included in the table.

It is true that the differences in the cardiac area when the vagus was stimulated before and after pericardotomy in some instances were slight. On the other hand, without exception the results all lie in the same direction. Most of the animals showed an appreciable difference. It is, of course, impossible to say whether the increased amount of cardiac dilatation would be sufficient to injure the cardiac fibers. It probably does not.

It is also doubtful if any physiological condition would stimulate the vagus to the extent reported in these experiments. The results apparently show, however, that the pericardium may have a definite restraining action on the heart and, as in the case of cardiac dilatation from anoxemia, it is quite probable that in cases of extreme stress on the heart the pericardium may play a protective rôle.

CONCLUSIONS

1. Animals with the pericardium incised show a greater cardiac dilatation when the vagus is stimulated to cause cardiac standstill than do animals with the pericardium intact.

2. The pericardium restrains excessive cardiac dilatation caused by vagal stimulation which is further evidence for the protective action of the pericardium in conditions of extreme stress on the heart.

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THE EFFECT OF INTRAVENOUS INJECTION OF CALCIUM LACTATE UPON GASTRIC SECRETION

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Sereghy and von Gyurkovich (1927) reported that intravenous injection of calcium salts is followed by an increase in gastric acidity and motility. The experiments were performed on human subjects, the methods of fractional gastric analysis being employed. The rise in level of blood calcium was regarded by these authors as causing a preliminary vagus stimulation strong enough to mask a subsequent sympathetic action with which calcium ions are usually considered to be associated. This claim of Sereghy and von Gyurkovich, that intravenous injection of calcium salts promotes gastric secretion, should be investigated further before being accepted. The object of the study here reported was to determine, in dogs having an isolated pouch of the fundus portion of the stomach, the effect on gastric secretion of intravenous injection of calcium lactate.

EXPERIMENTS. *Technique.* Five pouch (one Pavlov and four Heidenhain) dogs were prepared. At least a month was allowed to elapse between the time of operation and the beginning of experimental work with the animal. The diet and general scheme of feeding was essentially that used in this laboratory and described elsewhere (Cowgill, 1923).

In this investigation the level of blood calcium was raised by intravenous injection of a 5 per cent solution of calcium lactate. Blood calcium was determined by means of the Clark-Collip (1925) modification of the Kramer-Tisdall method. The withdrawal of 10 cc. of blood does not appreciably affect the level of calcium in the blood (Billigheimer, 1922).

Gastric juice was tested for free and combined acid by titrating first to the turning point of Töpfer's reagent and then to that of phenolphthalein.

PRELIMINARY EXPERIMENTS. *Determination of suitable dosage of calcium lactate.* It was first of all necessary to determine the amount of calcium lactate solution that could be administered intravenously without untoward effects. Preliminary trials were therefore made on a non-fistulous animal. In the first experiment a dose of 0.2 gram of calcium lactate per kilo of body weight—28 mgm. of calcium per kilo—was injected. Twice after the injection—after seven and twenty minutes respectively—

the dog showed tremors and vomited. Determinations of blood calcium were made before and at intervals after the injection. The calcium level rose to a maximum of 23 mgm. per 100 cc. of serum, found in the first sample taken 15 minutes after the injection, and fell to 15 mgm. in 117 minutes, or approximately two hours. In view of this result a second experiment was performed using a dose one-half as large. This dose—13 mgm. of calcium per kilo—produced a maximum rise of blood calcium to 17.6 mgm. per 100 cc. of serum. This amount was considered satisfactory for subsequent experiments. In their trials with a preparation of the parathyroid hormone, Austin and Mathews (1927) produced increases of the blood calcium level of about this degree. It is an interesting fact that this dose is identical with that used by Sereghy and von Gyurkovich, if one assumes that their human subjects weighed 70 kilograms.

Experiments on gastric pouch dogs. Preliminary trials showed that it was not advisable to attempt to determine the levels of blood calcium at intervals following the injection of calcium salt at the same time that gastric secretion was being observed. The process of withdrawing blood for analysis disturbed the animal, it being necessary to place the dog in a reclining position each time that blood was withdrawn. Collection of juice was felt to be less accurate under such conditions. Furthermore, there was the possibility that the discomfort incident to venepuncture might result in some degree of inhibition of the gastric secretory process. At least two separate experiments were therefore made with each dog in order to determine the effect on the blood calcium level of the intravenous injection of the dose of calcium lactate decided upon. Although the responses in two such studies were not identical, in every case a definite and significant hypercalcemia occurred. This was followed by a gradual fall in blood calcium level to slightly above normal in from three to six hours.

Intravenous injection of 0.1 gram of calcium lactate per kilo of body weight, an amount sufficient to produce a non-toxic hypercalcemia, does not promote the flow of gastric secretion in a Pavlov or a Heidenhain pouch dog. All of the ten experiments yielded negative results. In order to show that the experimental conditions were satisfactory for demonstrating any increase in the rate of gastric secretion, histamine was injected subcutaneously as a final control procedure, and in every case this was followed by a prompt flow of clear juice containing free acid. Illustrative data are presented in tables 1 and 2.

Discussion. The data might perhaps be interpreted to indicate that a hypercalcemia of the degree present in these experiments exerts a transient inhibitory influence on gastric secretion. However, in view of the fact that all volumes of secretion were small except those obtained subsequent to histamine administration, and slight errors in the collection of such small quantities of juice could be responsible for the slight decreases noted, we hesitate to conclude definitely that such an inhibition occurs.

In their study of the effect of the parathyroid hormone on gastric secretion Austin and Mathews used histamine in order to secure profuse secretion of gastric juice. Inasmuch as the object of the present study was to test the efficacy of hypercalcemia *per se* as a stimulus for gastric secretion, the use of histamine would seem to be contraindicated except as a final control procedure in experiments where the hypercalcemia was ineffective.

TABLE 1

Protocol of experiment 24

Dog E, Heidenhain pouch, weight 14.1 kilos

TIME	DURATION OF PERIOD	GASTRIC JUICE		REMARKS
		Amount	Rate of flow	
	minutes	*cc.*	*cc. per hour*	
10.35–11.20	45	0.50	0.67	"Normal." No free acid
11.20–12.05	45	0.35	0.47	No free acid
12.05	Injected 28.2 cc. of 5 per cent calcium lactate via jugular vein			
12.05– 1.05	60	0.25	0.25	No free acid
1.05– 2.05	60	0.30	0.30	No free acid
2.05– 3.05	60	0.55	0.55	No free acid
3.05	Injected 0.4 mgm. per kilo histamine subcutaneously			
3.05– 3.35	30	3.7	7.4	Latent period about 13 minutes. Free acid present

TABLE 2

Protocol of experiment 26. Effect of intravenous injection of calcium lactate solution on level of blood calcium

Dog E, Heidenhain pouch, weight 14.1 kilos

TIME	INTERVAL AFTER Ca INJECTION	BLOOD CALCIUM*
	minutes	*mgm. per 100 cc.*
9.55		10.6
10.36	Injected 28.2 cc. calcium lactate solution via jugular vein	
10.49	13	18.5
11.39	63	15.5
1.05	149	14.3
2.30	234	11.7

* Duplicate determinations.

Two stimuli may not only give a summation but may potentiate each other. For this reason histamine was employed in our experiments only as a final control stimulus. Again, should the increase in concentration of blood calcium have a slightly stimulating effect on gastric secretion, this might be masked by the more vigorous histamine response. An example of this is seen in the work of Austin and Mathews with four Pavlov pouch dogs. With two of their animals it was noticed that when a histamine stimulus

of 1 mgm. histamine dichloride was used and there was "maintenance of a proper water balance," the hypercalcemia did not enhance the secretagogic effect of the histamine; however, in response to the injection of one-half this dose of histamine, there was a marked increase in both free and combined acidity associated with the highest level of blood calcium. This seemingly positive effect of the parathyroid-induced hypercalcemia on gastric secretion was not regarded seriously by Austin and Mathews, because the responses to histamine as a whole were lowered after the injection of parathyroid hormone. This was attributed to the water intake being insufficient "to maintain the proper water balance of the dogs." The animals receiving the larger dose of histamine showed no effect whatever after the injection of the hormone, but during the course of the experiment they were given 125 cc. of water. The two sets of experiments just cited are difficult to compare: they do not seem to have been so planned that in each case but one variable was operating. One disadvantage of the technique used in the present study, namely, the small volumes of gastric juice, was overcome by Austin and Mathews by the use of histamine. On the other hand such use meant the introduction of another variable. Inasmuch as many workers have shown that the acidity of different samples of gastric secretion more or less definitely parallels the volumes collected, the fact that these volumes were too small to allow satisfactory quantitative study of acidity is without serious import. The appearance of free acid only after the histamine injection further emphasizes the negative influence of hypocalcemia *per se* on gastric secretion.

SUMMARY—CONCLUSION

Five gastric pouch dogs were prepared. The normal rates of gastric secretion and the physiological level of blood calcium were determined for each animal. One-tenth of a gram of calcium lactate per kilogram of body weight—13 mgm. calcium per kilo—was injected intravenously and the effect of this dose upon the level of blood calcium and on the rate of secretion of gastric juice studied separately. In every case a marked hypercalcemia unaccompanied by obvious untoward effects was produced. However this effect was transient, lasting from three to six hours. All of the variations in gastric secretion rate accompanying the change in level of blood calcium were considered to be within normal physiological limits. The final control response to histamine showed that in every case the experimental conditions were satisfactory for demonstrating the secretion of gastric juice in response to an adequate stimulus. The hypercalcemia produced in these experiments was not an adequate stimulus. This study does not confirm the work of Sereghy and von Gyurkovich.

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DIFFERENTIATION OF AXON TYPES IN VISCERAL NERVES BY MEANS OF THE POTENTIAL RECORD

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In previous investigations of "involuntary" nerves (Heinbecker and Bishop, 1929) it has been observed that certain groups of fibers in a given nerve trunk which differ in conduction rates, also differ characteristically in such properties as threshold, chronaxie, refractory period and duration of axon potential response, or time to maximum of potential at the stimulated point. A more systematic study of these properties in the fiber types of certain representative nerves is here reported, together with histological findings on those fiber groups with which can be associated more or less specific differences in the above properties. The object of this research is to study different types of axons in the same nerve, for a comparison of their properties under conditions as nearly uniform as possible for the different axons. It is not usually feasible to study all properties of all axons in one and the same preparation, owing both to deterioration of the preparation with prolonged activity and to the fact that any one nerve is in general unsuited to the investigation of all features of interest. Enough different factors can be investigated in each of several similar nerves, however, to insure a fair degree of correlation between the different properties of the different axon types observed.

Terminology. The nerves studied showed in the records of conducted potentials either two or three of the main elevations which have been designated *A*, *B*, and *C*, each of which may consist of several waves. These separate out due to differences of conduction rate. When various other properties, such as refractory period and duration of potential response, of the axons giving rise to these potentials are studied, it is found that those of the various subdivisions of the *A* elevation are closely similar, but are quite different from those of the *C* elevation. In the *B* division, however, the situation is more complicated. Certain nerves show a *B* potential characterized by a relatively fast conduction rate, others give one with a relatively slow rate, still other nerves have both, and then the two components of the *B* elevation may be either separated out or almost completely fused. Other properties than conduction rate, however, indicate

that the two components of this elevation are wholly different in type, the first component closely resembling the *A* type, the second the *C* type of these nerves. By means of various techniques, therefore, at least *four components* of potential can be differentiated, corresponding to at least four groups of nerve axons. Two, three or four recognizable components may be present in different combinations to contribute to two or three of the main elevations, *A*, *B*, and *C*. The first component is usually absent from the involuntary nerves studied. That is, in such nerves the *A* elevation is absent. The fourth component is *always* present, as the *C* elevation. The second and third components giving rise to the *B* elevation are of variable magnitude from nerve to nerve.

Evidence will be presented below that the four groups of axons which give rise to these four components of the potential record can be distinguished anatomically, at least to this extent; that we can say that each fiber group is *typified* by a recognized element of the nervous system. For instance, the first or *A* group is typified by motor and sensory fibers of skeletal muscle reflexes, etc., in the somatic system. The second or B_1 group is typified by the visceral afferents running in sympathetic nerves. The third and fourth groups, B_2 and *C*, are typified by the myelinated and unmyelinated motor fibers, respectively, found in "autonomic" nerves. It cannot be said that other axons than these do not contribute to these components of potential, nor that other components of potential or groups of axons do not exist. For instance, unmyelinated afferents may occur in certain of these nerves; but if they do, they presumably have the same properties as the unmyelinated efferents, i.e., *C* properties. The striking differences in properties occur between the two parts of the *B* elevation, that is, between the second and third components, which fact apparently separates the somatic type and one visceral afferent type of fibers on the one hand from the strictly autonomic type, myelinated or unmyelinated, on the other.

In a preliminary report of this work (l. c.), owing to the similarity in properties between the first two components as stated above, we have referred to the second component of potential as if it were a part of an *A process*, although perhaps separable from a first or *A elevation*. We here refer to this second component as B_1, to be not inconsistent with the terminology of Erlanger and Gasser (1930), who have found the limits of conduction rate for the *B* process in warm-blooded somatic nerves to be about 30 and 10 meters per second. They find that the *B* elevation in somatic nerves studied is not confluent with either *A* or *C* at sufficient distances of conduction; in the autonomic nerves which we have observed this distinct separation in conduction rates does not usually occur, and we have taken the lowest trough between elevations as the demarcation between *A* and *B*, etc. This criterion, as well as the rate of the start of the *B* elevation in these nerves from which the *A* is absent, leads to a wider range for the *B* elevation, 35—3 meters per second, the two components of this elevation overlapping at about 12 meters per second. We, therefore, find other

criteria than conduction rate more useful in judging of the character of the fibers present in autonomic nerves. The B elevation of somatic nerves thus falls *within the range of that* of autonomic nerves; for the warm-blooded nerves studied by Erlanger and Gasser it appears to fall within the range of the B_1 component of autonomic nerves, and refractory period measurements we have made on the saphenous indicate that this is correct. In the frog sciatic, on the contrary, both Erlanger and Gasser's conduction rate figures, and refractory period measurements of our own indicate correspondence with the B_2 component of the autonomic nerves of the turtle, although we find that a small B_1 component may sometimes be detectable in the frog sciatic.

The only reinterpretation of previous statements required is to read B_1 for A in our previous communication, wherever the conduction rates are less than 30 meters per second for mammalian, or 15 meters per second for cold-blooded nerves, understanding that in the differential properties discussed, there is no great difference to be detected until we come to the B_2 component, there referred to simply as B. We find that in the nerves we have studied, the A and B elevations as here designated, as well as the two components, B_1 and B_2, tend to overlap, precise limits of conduction rates being impossible to assign.

I. PROCEDURE. In general, the correlation between histological structure on the one hand, and on the other, that group of properties recognized by means of the nerve potential record, is tested by the following procedure. The conducted action potential records of various nerves are observed on the oscillograph, and the conduction rates and relative magnitudes of the different groups of waves characteristic of each are noted. In the histological cross section of each nerve, the relative areas occupied by fibers of different types are also noted. In certain nerves, one or more fiber types are relatively poorly represented or lacking in the histological section, and in these cases, one or more components of the total possible potential are also found to be inconspicuous or absent in the oscillograph record. The correspondence between the elements lacking in the two pictures, even more than that between the elements present, furnishes evidence as to which fiber type gives rise to which potential. The other properties of the fiber types present are then ascertained by means of the action potential record as described below.

It is perhaps needless to say that generalization based on the findings in these few nerves which would apply to the nervous system at large, would be based only on inference from the part to the whole. Such generalizations at the present stage of this investigation would only be useful as working hypotheses, and no inclusive statement is intended at present as to other nerves. We presume that the correlations between the groups of properties so far studied represent the rule in the involuntary nervous system of both cold-blooded animals and mammals; on the other hand other types of axons not observed by us may have other groups of properties. The work here reported was not designed to throw any light upon the

physiological function served by fiber types in the body, although certain inferences may be drawn in this connection also.

Apparatus. Galvanic currents, condenser charges and induction shocks have been used to stimulate, but for the purposes of this research induction shocks have proved less suitable than condenser charges. To facilitate the procedures required, a stimulating apparatus has been arranged with which galvanic currents or condenser charges may be applied, either singly or repeatedly, or two stimuli, to be spaced at any required short interval. This apparatus is diagrammed in figure 1.

Dial 1 in the left hand tier delivers potentials by fifteen 1.5 volt steps, from flashlight batteries, up to 22½ volts; this bank, or the fraction selected, is in series with the contact arm of dial 2, and thus adds fractional steps of the larger steps of dial 2. Dial 3 is in parallel to dial 2, point for point, with steps of 1.5, 3, 3, 4.5, 4.5, 6, and 6 × 22.5 volts, giving a total for dials 1 and 2 of 157.5 volts and for 3 of 135 volts. Dial 4 selects pairs of condensers ranging from 0.0002 to 0.1 mf. for pairs of stimuli, in measuring the duration of the refractory phase. Dial 3 serves as the source of the first of two stimuli for refractory period measurements; dials 1 and 2 give the second stimulus adjustable by steps of 1.5 volt; and by selection of condensers, the quantity of current delivered per 1.5 volt step may be made as small as necessary, the smaller condenser giving a smaller difference of current per step. S_1 and S_2 are tap keys that lock down when turned; with the interruptor going, these keys can be depressed during one cycle only for a single pair of stimuli. S_3 is a similar key acting as a short circuit. R is a pair of leaks across the condensers such that the latter are discharged between interruptor contacts, and high enough (0.5 to 5 megohms) so that current through them and through the nerve is insignificant. They can also be so connected that no current flows from them through the nerve. In the output is a pair of resistances of 100 ohms each, insignificant in comparison to the nerve resistance, but effective in preventing high current flow through the short-circuit key S_3 or through the interruptor contacts.

On the right hand side, the three dials C_1, C_2, C_3 give thousandths, hundredths and tenths, respectively, of a potentiometer of 200 ohms total resistance, for measurements of chronaxie. It is operated by opening a short circuit across the output by means of the interruptor, during an adjustable time interval in each cycle; also controlled by the short-circuiting tap key in parallel with it. ABC is a three-way selector switch, passing condenser charges, galvanic current from the potentiometer, or, in the middle position, galvanic current from the potential selector of the left hand side of the box. Finally by a connection on the face of the box, potentials from the right hand potentiometer can be led through a condenser in dial 4 and thence to the nerve, for fine gradations of strength of stimulus.

Batteries are inside the metal box, which is grounded as an electastatic shield, and all leads are shielded. The tap keys have platinum contacts. All switch points are heavily smeared with vaseline. Currents are timed at intervals determined by a rotating interruptor, or by the pendulum interruptor previously described (Bishop, 1929) and the resultant potentials observed on the oscillograph after amplification up to 200 millimeters deflection per millivolt.

Leading into an amplifier grid circuit, the current drawn in taking the nerve record is so slight that metal lead electrodes are not appreciably polarized.[1] The stimulat-

[1] The condition of least current for a vacuum tube input is that the grid bias be placed at the potential value which the grid would assume, if its circuit were opened. This potential is determined by the tendency of the grid to accumulate or lose elec-

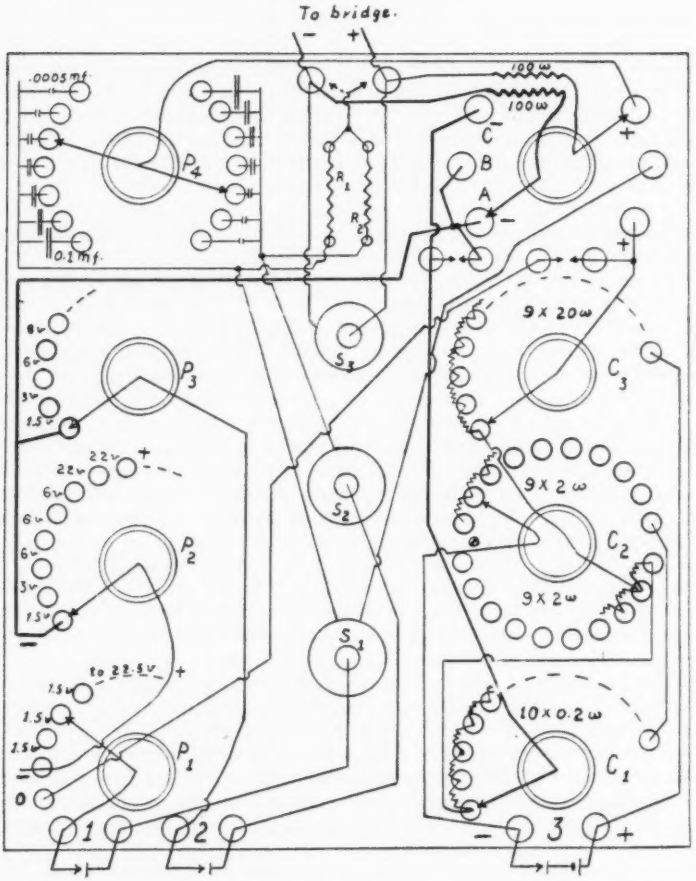

Fig. 1. Stimulating apparatus, employing banks of dry cells and potential selectors to give two stimuli as condenser charges, or galvanic currents from a 1000 step potentiometer. Details in text. In dials *1*, *2*, and *3* the contact arm is raised while passing from one contact to the next to avoid short-circuiting the batteries.

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trons or positive ions, and is usually more positive (−0.5 to −1.0 volt wth respect to the filament) than the customary −1.5 bias. However, since the change of voltage of the grid of the first tube of an amplifier is very small, the bias can safely be put at the value of zero grid current (determined for a given tube as that value which will permit of opening the grid-ground circuit without a change in the output). A −1.5 volt bias may result in a current of 5×10^{-8} amperes.

ing electrodes, however, must be nonpolarizable, especially with the high sensitivity of apparatus required for recording potentials of involuntary nerve components, and with the strong stimuli required to activate them; otherwise the stimulus escape becomes unmanageable. We have used calomel-Ringer half cells with yarn wicks, or silver-chloride electrodes.

Where stimulating and lead circuits are grounded separately, electrodes of two different types are not used, as large potential differences are then set up along the nerve.

A convenient form of silver electrode has been constructed as follows (fig. 2). In a block of hard rubber three narrow saw cuts are made longitudinally, and one transversely near one end. Each longitudinal cut houses a coil of silver wire immersed in Ringer's solution. The transverse cut tapers, narrowing toward the bottom, to accommodate nerves of varying diameter, and by its wedge form, holds one end of the nerve firmly. The wire *1* proximal to the lead electrodes is connected to the ground

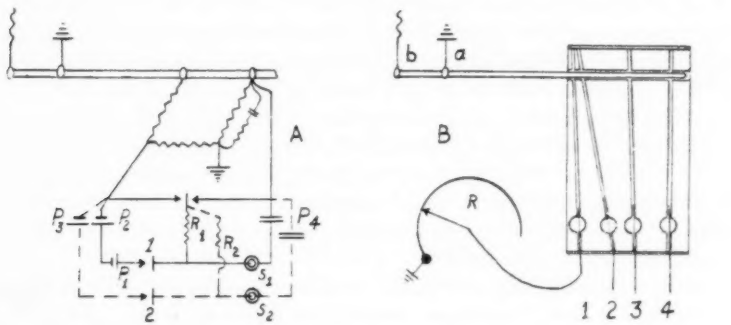

Fig. 2. A, Diagram of circuit for two condenser stimuli. Lettering as in figure 1. B, Stimulating "pore" electrode. Description in text.

through a variable high resistance. Slot *2* is the cathode, slot *3* or *4* anode of the stimulus. By varying the resistance from *1* in series to ground, the stimulus escape can be largely eliminated.[2] The ends of these longitudinal slots are closed with DeKotinsky cement, which at one end holds the wires in place. If the outside of the block is vaselined, fluid will not run out along the nerve, nor short-circuit across the top. The wires are electrolysed *in situ* in Ringer's solution to reduce polarization.

A further convenience develops in connection with this electrode, in that, since the nerve resistance is not very different from that of Ringer's solution, and since the nerve cross section is a small fraction of that of the slot containing it, approximately the same current flows from a given stimulus whatever the diameter of the nerve. The potential drop from cathode to anode therefore remains about the same for nerves of different size. The result is that even in nerves of widely different diameters the thresholds as measured, of fibers with the same properties, tend to be approximately the same, provided, of course, that the temperature and other conditions remain constant. If, moreover, the end of the nerve lies in the slot *5* at a point

[2] The reason for the effectiveness of this arrangement has not been investigated in detail, but is apparently due to the circuit forming an effective bridge network, one ground lead compensating the other.

between *3* and *4*, and *4* instead of *3* is made anode, the current tends to flow off the nerve at the killed end, rather than transversely through the side, reducing the tendency of the nerve to respond at the anode as well as at the cathode.

Recording. It is found in general that the different waves of the potential record are easier to detect if the record is somewhat diphasic; a condition that will result the more completely, the nearer the killed point is to the distal lead electrode (Bishop, Erlanger and Gasser, 1926; Heinbecker, 1929; Gerard, 1930). Most of the work was done on linear time coördinates, obtained by charging the condenser in parallel with the X axis deflecting plates of the oscillograph, through a vacuum tube at current saturation (Bishop, 1929) the time being varied by varying the condenser or the filament current of the charging tube. Time was recorded by means of an electrically driven tuning fork, which may be set somewhere near, but not connected to the apparatus. Sound oscillations may be mechanically picked up as sine waves, or the break of the current driving the fork may be radiated electrically as a series of shock-like deflections. Time can then be measured on the face of the oscillograph with dividers, or recorded photographically by a single deflection of the oscillograph beam; if the movement is too rapid for single deflection recording, a larger condenser is thrown in, the rates of movement determined by the two condensers, with constant current through the vacuum tube, being inversely proportional to the capacities.

All nerves, both cold- and warm-blooded, were supplied with oxygen and stimulated slowly (1 to 10 per second) and the results were checked with single stimuli after periods of rest.

Sources of technical error. Several factors have appeared in this and previous work which, if not obviated, are apt to compromise the accuracy of the observations here reported. First, the differences of threshold for different fibers are so great that, for instance, prolonged stimulation of C fibers may result in depression of irritability or even in death of the A fibers at the stimulated point. In a given nerve, therefore, work on the fastest conducting waves is finished, where the object of the experiment permits, before the stimulus is raised to bring in the later ones. The last fibers may respond only to stimuli 100 or more times that adequate for the first. On the other hand, the slower fibers fatigue with stimulus rates that the faster fibers will respond to without reduction in amplitude; this fatigue shows up as a rise in threshold, prolonged refractory period, slower conduction and lowered amplitude at maximal strength of stimulus. It is not confined to the region of the stimulus, therefore.

Second, it is impossible to obtain even approximately monophasic records of the late waves, without killing the nerve back so closely to the proximal lead electrode (two or three millimeters) that the amplitude of potential is materially depressed by spread of the injury back to this electrode. Even

here the record is probably not truly monophasic; the two phases are merely closely superimposed, the second being the more depressed in amplitude, and thus being overpowered by the first. When the nerve is killed under one electrode, the dead nerve apparently acts as an extension of the electrode contact to live nerve, which in these fibers, quite as in the heart, still permits of a considerable potential being recorded as from that point. The condition is an accentuation of that previously discussed for the *A* wave of the frog sciatic (Bishop, Erlanger and Gasser, 1926).

Owing, moreover, to the slow conduction of these late waves (of the order of 1 M. per second) the time elapsing between the passage of the wave at the proximal electrode and its arrival at the killed point, even though this distance be but a few millimeters, is sufficient to allow considerable separation out of the two phases. Completely diphasic records are therefore preferable when circumstances allow, and it is often possible to place the two lead electrodes both on live nerve, and so far apart that the two phases are entirely separate; each phase is then a "monophasic" record. The complicating factor here is that the second phase of, for instance, the *B* elevation is liable to superimpose upon the first phase of the *C*, etc., and since both these elevations are often double, complicated figures may arise, for in many cases sufficient lengths of nerve cannot be obtained to allow of complete separation out with conduction of all the various elements of the record. A few fortunate exceptions to this situation will be noted below.

Third, with the strength of shocks (condenser charges) necessary to stimulate the slow fibers, high and long lasting polarization of the nerve under the stimulating electrodes apparently takes place, which may occasion a serious distortion of the base line throughout even a very slow record (Bishop, 1929). This can be largely compensated by making the section of nerve between stimulating electrodes one arm of a Wheatstone bridge (Bishop, 1927) but without elaborate and precise compensation in the other arms, this leaves still a considerable shock distortion overlapping the first wave. Since the first and third elevations cannot be recorded satisfactorily on the same time scale, this is not usually serious, as a lower stimulus will allow a return of the shock deflection approximately to the base line before the *A* process is recorded. Such long-lasting deflections are much less pronounced, and thus easier to compensate, in nerves of small diameter, and are, of course, less prominent, the further the lead from the point stimulated. They are easily differentiated from action potential "retentions" by observing the shock potential form below threshold for a given component.

Finally, for the stimulation of the more slowly conducting fibers, long lasting currents are almost necessary. With charges from a 0.01 mf. condenser, 100 volts may be insufficient to elicit all of the main *C* wave, in a nerve where 1.5 volts through a 0.002 mf. condenser stimulates the larg-

est A fibers. Using larger condensers (0.02 to 0.1 mf.) the disparity is not so great; for comparative readings of thresholds the same medium capacity is used throughout. It should be possible using a graded series of condensers to characterize the different types of fibers by two parameters, voltage and capacity, determining the optimum values, i.e., the combination by means of which the threshold is reached with minimal energy for each fiber type. This has not been done, and values in terms of voltage across a constant capacity are perhaps more useful as criteria for practical application of the information as to thresholds of different fibers in a given nerve. Moreover, this optimum condition being related to chronaxie (Lapicque, 1926), the information is derivable from chronaxie values given. However, it should be recognized that different methods of stimulation (i.e., condenser charges as compared with induction shocks or galvanic currents) would give different ratios for threshold values of the various potentials. Nerves of different sizes, having different resistances, should then exhibit variations in relative stimulus strengths, due to the opposite effects of resistance on the time course of the discharges of condensers and inductances.

Duration of potential. We have attempted to record the durations of the potential responses at the stimulated point, for different axon types, by the same technique as used heretofore (Bishop, 1927), but without such precise results as may be obtained for more irritable fibers. The sensitivity of apparatus required for recording the late potentials of nerve (twenty or more times as great as for the frog sciatic A wave) together with the stronger stimuli required to elicit them (five to fifty times as great) render the shock escapes when leading directly from or even close to the stimulating cathode, relatively enormous. It has been technically impossible so far to balance out completely in a Wheatstone bridge a shock of the order of one hundred volts, when leading into an apparatus with a sensitivity of one to two hundred meters deflection per one volt. The rising phases of the resultant action potentials are thus somewhat distorted, although it has been possible by this technique to determine the order of the duration of the B potentials as compared to the known values for the A. The B_1 potential of the turtle has a time to maximum but little longer than that of the turtle sciatic (0.7 and 0.5σ respectively) and in the cat sympathetic, the B_1 time is slightly longer than that reported by Gasser (1928) for the dog phrenic A (about 0.3σ as compared to 0.2σ). It is further certain that the B_2 component lasts longer than the A by a factor of at least four or five, in both turtle and cat. The C potential lasts considerably longer than that of the B_2 component, possibly twice as long.

Two other procedures have been employed to confirm these direct findings, but without appreciably greater precision. In the first, two stimuli were sent in over the same electrodes, the second within or shortly following the absolutely refractory

period of the B_1 process arising from the first, the nerve lacking an A process. The first was maximal for B_1, the second was stronger to give B_2. Two to three millimeters conduction was allowed, to obviate the large escape, the stimulating circuit being also in a bridge. If it be assumed that the different axon processes of the B_2 component separate out with conduction to the same extent *relatively to their duration* as do those of the B_1 (for which we have no proof), the *relative* durations as observed after conduction will be valid for the stimulated point. At any rate no great error is introduced in a short conduction distance, compared with the magnitude of the observed differences, and with the other sources of difficulty.

The third method consists in leading, diphasically, from two electrodes far apart and both on live nerve. If the distances are great enough, and if the nerve is simple enough in fiber content, the difference in the durations of the two phases can be extrapolated back to zero conduction distance, since the difference is only due to the different conduction rates of the constituent axon potentials summing to form the potentials at each lead. These will be broader and lower, the further the conduction. A nerve must be chosen which has little or nothing conducting ahead of the wave to be measured, one which is uniform and without branches, and which is long enough to allow the first phase to approximately recover at the electrode proximal to the stimulus, before the impulse reaches the second electrode; it is also necessary to leave at least one centimeter of nerve suspended beyond and free from the distal electrode. The nerve is stimulated far below maximal, to avoid later potentials than those being observed, and also because, even within one group, the elevation becomes complex with conduction, the successively slower elements having successively higher thresholds. Obviously, injury to any considerable number of axons would vitiate the experiment by rendering the two phases of the potential incomparable.

In spite of slower conduction, which might be expected to allow of better separation out of the two phases of the late potentials than of the earlier, the distance of conduction necessary for such separation is considerable, because the axon action potentials are long in duration in correspondence with their slower rates. Since

$$\text{rate} = \frac{\text{wave length along nerve}}{\text{wave duration at a point}},$$

the length of nerve active at one time, rate *times* duration, tends to be more nearly the same for different types of axons than it would be if all had the same duration. Data are not considered sufficiently precise as yet to render profitable the computation of temperature coefficients, for the significant comparison of warm- and cold-blooded nerves. The latter are, however, much longer in duration in the turtle at 25°C. than in the cat at 37°C. The data we have indicate at least the order of the durations of potentials in the nerves studied.

Absolutely refractory period measurements appear at first simpler and less equivocal, the chief precautions necessary being that the nerve be unfatigued and uninjured in dissection, and that the rates of stimulation be very slow. This applies to the faster conducting elements of the nerve as well as to the slower. The refractory periods we have obtained consistently for the A and B_1 processes are about as short as the shortest of those previously reported (Erlanger, Gasser and Bishop, 1927), but larger values are obtained if stimulation is too rapid or too prolonged, and the slower conducting fibers are the more affected. Our values check satisfactorily with the recent results of Heinbecker (1929) and of Amberson and Downing (1929).

One rather surprising result has appeared that we have not investigated sufficiently to attempt to analyze. The refractory periods of the B_2 and C axons may apparently be as short as their respective times to maximum. What this means for the potential at the stimulated point it is difficult to say; it would suggest, if valid, a summation of potentials in the same axons, which fused potentials would then separate out with conduction. This seems at first improbable; but a similar thing happens to a lesser degree in the A potentials, where a second stimulus applied before the complete subsidence of the first potential response (in fresh nerve) results in a second

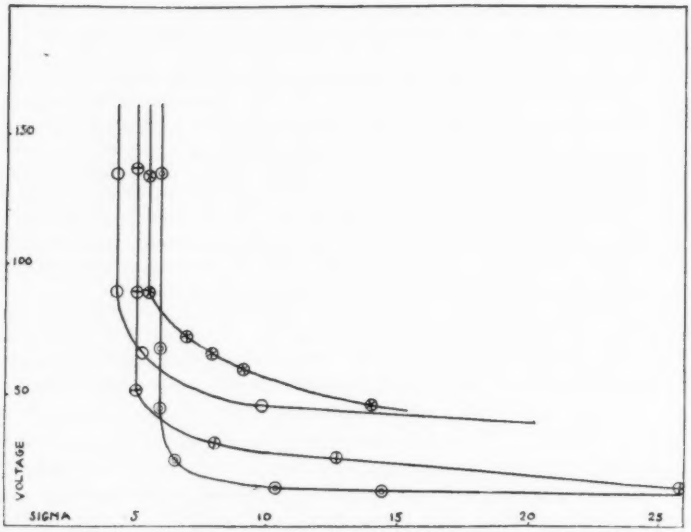

Fig. 3. Recovery curves, for second response, main C wave turtle sympathetic and vagus nerves, to determine absolutely refractory phase. Other values are larger. See legend, figure 5, and text.

potential wave rising from the falling phase of the first. The implications for the validity of the all-or-none law as applied to these fibers need not be considered until the finding itself is further studied, but the analogy of such a condition in smooth muscle might properly be suggested, since smooth muscle is presumably innervated by these fibers. The situation cannot be studied at the stimulated point because of the huge shock effect from the strong second stimuli required.

Refractory periods are determined by observation of the response to the second of two stimuli, after the potentials have separated out with conduction. The C elevation becomes of such long duration after conduction.

when the first stimulus is maximal, that such separation is usually not complete for this process in lengths of nerve available. The first stimulus will activate even the slowest fibers, while the second will presumably activate only the fastest, since these will in general have the lowest thresholds. These two elements therefore tend to superimpose, and what we see is usually an increase of amplitude or a prolongation of the falling phase of the first response, instead of a discrete second wave. For this reason we have plotted the curve of return of irritability after the first response, to assure ourselves at least that the limit of that curve, which is the end of the ab-

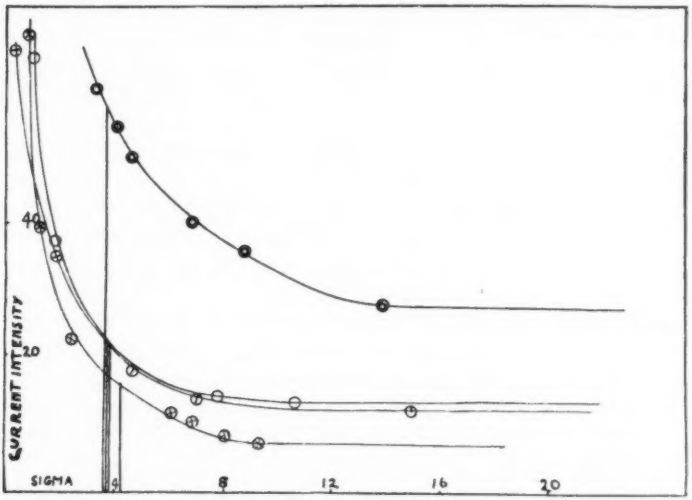

Fig. 4. Chronaxie curves, for start of main *C* elevation, turtle sympathetic and vagus nerves. These are consistent low values. Other scattering higher values we infer to be assignable to depression of the particular nerves.

solutely refractory phase as observed, is continuous with the curve itself. This does not totally eliminate an error of reading, because the error might increase gradually as the curve approaches its limit. The times between stimuli can be read with great precision by speeding up the movement of the oscillograph beam far above the rate suitable for observing the slow responses. Typical values are given in table 5 with curves in figure 3.

Chronaxie measurements were made by a technique similar to that for refractory phases, galvanic currents being timed by a double or make-break contact wheel, and intensity controlled by a potentiometer, the threshold response being observed after conduction. Again the whole curves were plotted, and the values of chronaxie read from the curves. It is

significant to observe here that the rheobasic current strength values for fibers far apart in chronaxie are disproportionately close together, the difference being chiefly in the duration required for threshold current to stimulate. Values for typical nerves appear in table 5 and figure 4.

2. Correlation between properties of the axons of a complex nerve trunk. Cold-blooded nerves. As a type nerve for this account, we may select the turtle cervical sympathetic trunk analyzed by Heinbecker (1930a). In this nerve the A elevation is always absent; the nerve further differs in different turtles by the presence or absence of the first part of the B eleva-

POTENTIAL COMPONENTS	A VALUES FOR FROG	B_1	B_2	C
Rate, m.p.s.	(45)	16–8	4.5–3.0	0.8–0.3
Threshold arbitrary units, 20μ fiber assumed 100	(100)	300–400	2,000–3,000	5,000–15,000
Time to maximum at stimulus, σ.	(0.27) 0.5 + turtle	0.5–0.8	3.0–4.0	over 5.0
Chronaxie, σ	(0.3)	0.35–0.45	3.0–4.5	3.5–5.0
Absolutely refractory period, σ.	(0.8–0.9)	0.9–1.1	3.5–7.0	4.5–10.0

Fig. 5. Turtle autonomic nerve properties, average values or limits of observed values. The slower rates and higher values of refractory period, etc., may be assigned to depression in nerves removed from the body for some time. The shorter values of refractory phase may be too short owing to difficulty of obtaining a well-separated out second response in complex nerves at the conduction distances available. Our least equivocal experiments have given values within the higher figures designated. Temperature 20 to 25°C. Threshold of 2000 arbitrary for B_2 process, when B_1 is lacking. This table gives the order of the values, and rough limits for nerves in the conditions obtaining in experiment.

tion (fig. 3). When this component of potential is absent, the nerve lacks the larger myelinated axons, and there is reason to believe that this group of axons corresponds to the sensory fibers of the depressor nerve of other animals; it is probably sensory in part at least in the turtle. When it is absent from the sympathetic a separate nerve carries these fibers (Heinbecker, l. c.). These relationships identify the axons responsible for the B_1 potential histologically (see section 3 below).

The different elements of potential separate out from each other with conduction because of differences in conduction rates. With these are associated differences in threshold; the slower the conduction, the higher

the threshold. The B_2 component appears to arise from certain of the small thinly myelinated axons of these nerves, and the C wave from the unmyelinated (Heinbecker, l. c.). While the separation due to differing conduction rates is not always particularly sharp between any two given components of the potential, the interval of threshold between certain waves is more abrupt, and at these points there is still more abrupt and striking change in the duration of the absolutely refractory period, chronaxie and duration of the axon potential at the stimulated point. The most abrupt changes in these properties take place between the B_1 and B_2 components.

For instance, a B_1 component travelling 10 to 6 m.p.s. has a chronaxie of 0.3 to 0.4 sigma, approximately equal to that of the cold-blooded frog or turtle sciatic nerve fibers which travel 45 to 10 m.p.s., while the B_2 component travelling 3 to 4 m.p.s. has a chronaxie of 3.5 sigma, and the main C wave, 0.5 m.p.s. and 4 sigma. Absolutely refractory periods (minimum values) are 0.9, 3.5 and 4.5 sigmas respectively. The time to maximum for the A elevation of the frog sciatic (Bishop, 1927) is 0.27 to 0.3 sigma, at the stimulus, and for the turtle sciatic at least 0.5 sigma. For the B_1 component of the turtle sympathetic the time to maximum is about 0.7 sigma, while that for the B_2 wave of the sympathetic is five or more times as long, and for the C wave considerably longer still. It is thus obvious that the abrupt change in certain properties takes place between the B_1 and B_2 elevations rather than between B and C, and without an abrupt change in myelination of the axons. The vagus of the turtle gives potentials closely similar to those of the cervical sympathetic, the A elevation being lacking in this nerve also. Presumably the cervical branch, described by Ranson (1915) as coming off near the anterior ganglion, and carrying large fibers, would have an A process, as the cat vagus has. This region is anterior to that here considered. The vagus has usually more unmyelinated axons, as Ranson also notes, and correspondingly gives a relatively larger C elevation than does the sympathetic. Two turtle vagi have been recorded with *no* B_1 component in the region employed. The above author finds no evidence in the turtle that myelinated axons lose their myelin sheaths in their courses through these nerves, nor have we found anything that would indicate that the fiber groups are not specific and distinct, or that preganglionic myelinated ever synapse with postganglionic unmyelinated (Heinbecker, 1930b) at least in the turtle nerves under consideration.

A small wave follows the main B elevation closely in conduction rate (fig. 7, 3) lies between the second B and the main C elevations in threshold and refractory phase, and has been identified by Heinbecker (1930b) as being due to postganglionic fibers synapsing in ganglia proximal to the superior cervical. It is included provisionally in the B elevation as B_3

because the increase of stimulus necessary to elicit it is less abrupt than is the further increase necessary to bring in the later waves. Its properties lie between those of the B_2 and C elevations. It is present in the corresponding nerve of the cat, also in the vagi of both animals.

If we make the demarcation between B_1 and B_2 and B_3 and C as outlined above, it turns out that the fiber groups which can be differentiated by their other properties may have, in these nerves, conduction rates that almost overlap. In fact, if the stimulus is increased gradually while the growing picture is being observed on the oscillograph, it appears that some fibers which come in after the relative interval of stimulus increase that signalizes the appearance of a given component, have a rate faster than the last fibers of the earlier component, and thus there is not necessarily a distinct interval in conduction rates between different types of fibers.

POTENTIAL COMPONENTS	A	B_1	B_2	C
Rate, m.p.s. starts of major waves	80–50	35–15	15–10	2–1
Threshold, arbitrary units	150–200 (15–17 μ fiber)	300–400	1,200–1,600	4,000–10,000
Time to maximum at stimulating point σ	(0.2) (dog, Gasser, 1928)	0.3+	2.25+	3.5+
Absolutely refractory period, σ.	0.6–0.75	0.6–0.85	2.2–4.5+	4.0–6.0+

Fig. 6. Properties of nerves from warm-blooded animals. See legend, figure 5. T. 36–38°C.

Typical data on cold-blooded nerves are collected in the table, figure 5.

Warm-blooded nerves. In involuntary nerves from warm-blooded animals studied, the potential record is in general similar to that of the turtle sympathetic, a sharp transition occurring in certain properties between the B_1 and B_2 elevations, and a less sharp demarcation between B and C. In the cat sympathetic, the B_1 elevation with several maxima fuses with the B_2 (Heinbecker, 1930a) and yet the refractory period of the B_2 is four times as long as that of the last of the B_1 (2.25 sigma and 0.65 sigma, respectively, minimal values). In general all subdivisions of the A and of the B_1 components in different involuntary nerves have approximately the same refractory period (0.6 to 0.75σ) within the limits of observation in these nerves, quite as has been reported (Erlanger, Gasser and Bishop, 1927)

for the α, β and γ waves of the A elevation of the frog sciatic. The refractory period of the main C wave has been measured in the cat vagus, where the wave is usually larger than in the sympathetic, in both branches of the genito-crural, in the sympathetic and in the depressor, in all cases being between 4 and 6σ.

The properties giving rise to corresponding waves in the vagus and sympathetic nerves are closely alike, granting that the A process present in the vagus and absent in the sympathetic arises from the large fibers present only in the one nerve. One cat sympathetic has been observed (reported previously, Heinbecker, 1930a) in which only the first wave of the B_1 component was present (38 meters per second conduction rate). The significance of this was missed at the time, and the nerve was not elaborately studied. Since then in a second cat nerves have been observed in which little if any part of the B_1 component was detectable in either cervical sympathetic. On the right side a separate fine nerve ran in the vago-sympathetic sheath, and was dissected out for 50 mm. On the left side the middle portion of this nerve could not be freed from the vagus, but one free end could be stimulated and leads taken from the other. The free nerve was that referred to in the preceding paragraph as the depressor.[3] Both these fine nerves had one large wave, a small second one and a medium third (fig. 7, record 8) and each wave was well separated from the next by a practically vacant interval.

The first and second waves of these nerves appear to correspond with the B_1 and B_2 components of the more usual sympathetic nerves, except that these components were here more distinctly separated out than in the usual case. What appears to have happened is that the part of the B_2 component running in this depressor corresponds to the latter part of the B_2 component of the sympathetic (which is, in fact, usually double) and thus a relative gap is left in the position of the earlier part of the B_2 component. The B_1 component of the depressor would then correspond to that which in other animals courses in the sympathetic (and vagus). The very close similarity between the condition in this cat and in the rabbit supports such an interpretation, although it cannot be excluded that a separate depressor nerve may also carry fibers which otherwise course in the vagus. This cat depressor distributed fibers to the viscera peripherally, and joined the vagus trunk centrally. In this depressor nerve, the absolutely refractory period of the B_1 and B_2 components corresponded to those of the sympathetic B_1 and B_2.

[3] Doctor Gruber informs us that the depressor nerve is known to be very occasionally separate in the cat Unfortunately, this animal was past the stage where the appropriate experiments could be formed *in vivo* by the time the situation was recognized. It was observed, however, that anteriorly the depressor entered the *vagus* trunk.

It thus appears that most of the B_1 component, a specific late element of the B_2 component, and part of the main C group were here segregated from their customary position in the sympathetic into a separate "depressor" nerve. The rabbit sympathetic investigated, like that of this one cat, had no B_1 group, while the depressor had a large one, and the rest of the records from the rabbit nerves studied are precisely like those from this cat. The histological correlations are reported below. The parallel between these nerves and the turtle sympathetic when its B_1 component is absent is rather complete. The obvious inference is that the B_1 group of axons is sensory, and includes the specific depressor afferents; since the B_2 and the C fibers, in the turtle at least, all synapse in the sympathetic ganglia, while the B_1 do not.

Fig. 7. Action potential curves from mammalian nerves. Repeated stimulation, 1 per second. Approximately linear time, except records *9* and *10*. Reduced in reproduction to $\frac{6}{10}$ size. *1*, right vagus of cat, nerve killed at distal electrode but still diphasic, section of nerve in figure *9–1*. Small B elevation and large C corresponds roughly with relatively numerous unmyelinated axons. *2*, *3*, left vagus same cat, nerve killed at distal electrode, 53 mm. conduction, 12 mm. between leads. *4*, proximal lead moved toward stimulus; distance stimulus to first lead, 40 mm. between leads, 25 mm., killed only at end. *5*, same as *4*, except killed half way between leads. Killing close (3 mm.) to proximal lead left the potential still nearly as diphasic as in record *5*. The B_2 and C elevations are distinctly double in all records, with a potential preceding the main double C elevation. The second phase of one wave tends to obliterate the first phase of the following wave, except the first and last. By the time records *4* and *5* were taken, the earlier waves were much depressed by strong stimuli, compare record *2*. Note that in record *5* (shorter time between records of two phases, recorded at proximal electrode and at *killed point between* electrodes) the second downward phase starts earlier than in record *4*, indicating that it records at whatever region is killed. *6*, *7*, right sympathetic nerve of same cat as previous records. *8*, record of separate depressor nerve of this cat, B_1 component not detectable in sympathetic by refractory period measurements but present in depressor. Sections in figure 8, *1* and *2*. Nerves on opposite side corresponded exactly. *9*, cat genito-femoral, *10*, human genito-femoral. Refractory period measurements show abrupt change at vertical lines, from a value for A and B_1 components of 0.6–0.7σ to a B_2 value of 2.2-2.5σ. *11*, intestinal branch of coeliac plexus of cat, section in figure *9–4*, completely diaphasic, distance stimulus to proximal lead, 9 mm.; between leads, 7 mm.; for extrapolation to derive duration of potential (see text). *12*, same, killed just proximal to distal electrode, second phase depressed. Distance stimulus to lead electrode, 7 mm.; between leads, 10 mm. This nerve had a very simple C elevation that prolonged but slightly with conduction. *13*, B_1 component, same time scale as *11*, same amplification. *14*, same, recorded at twice time rate of oscillograph. The shock artefact is barely detectable as a break in the base line separate from the wave. Notice that fast component is here apparently nearly monophasic under conditions that gave the distinctly diphasic record *12*. At strengths of stimuli between those necessary for records *11* and *13*, a very slight elevation of the line following the first wave presumably comprises what little B_2 component this nerve possessed.

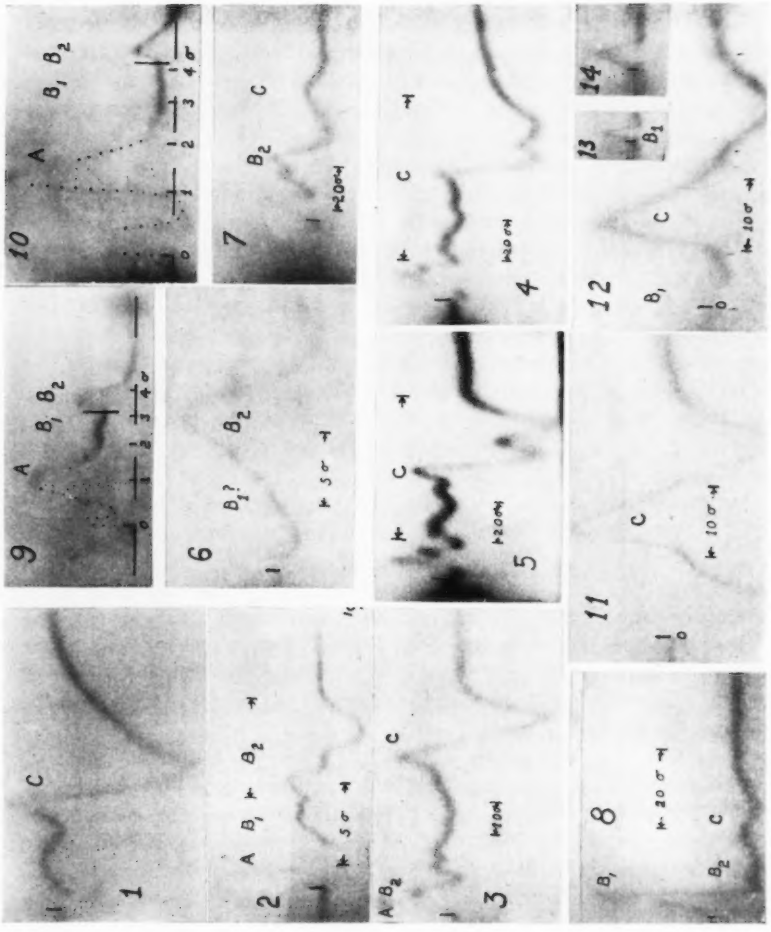

Fig. 7

In the genito-femoral nerve of the dog, cat, rabbit and human the conditions are closely similar to those in the involuntary nerves discussed heretofore. This nerve separates out into two branches, one going into the body wall direct and passing, in part at least, to the skin, and one passing toward the genital region. The genital division appears to carry out both somatic and visceral fibers. This genital branch has, in the dog, cat, rabbit and human nerves studied, a complex A elevation which appears to lack the first or α wave, but shows a β and a γ. The B_2 main wave may nearly equal the A in amplitude, and the C may be even higher. It thus serves excellently for differentiating between the various fiber types present, under uniform conditions, and with more or less the same degree of precision for the three waves all of comparable amplitude.

Between the first high waves in the A position and the main or B_2 wave, of this nerve (fig. 7, records 9 and 10) the record does not return to the baseline, and two or more minor waves appear, quite as a train of waves of decreasing amplitude occurs in sciatic nerves. In this case, however, we are inclined to assign the small waves to a B_1 category, by analogy with the involuntary nerves discussed previously. The absolutely refractory periods of the first high and following low waves lie between 0.6 and 0.7 sigma; that of the B_2 is 2.4 to 3.0 sigma, and of the C 4.5 to 6 sigma. The figures correspond within reasonable limits of variation to the values for the corresponding waves in the vagus and sympathetic nerves, and the demarcation between B_1 and B_2 components as detected by refractory period measurements is equally sharp. Certain large and heavily myelinated axons present here, but absent from most involuntary nerves, would be inferred on the basis of the work of Gasser and Erlanger (1927) on fiber size and conduction rate, to contribute to the first large wave of the A process. Other more thinly myelinated axons are similar to those usually found in the sympathetic.

Correlation of these and the previous data with the histological picture, therefore, leads us to the conclusion that the first large A elevation represents chiefly a somatic component of this nerve, motor or sensory; and that the B_2 wave is involuntary motor, in the small and thinly myelinated axons, while the C is unmyelinated.

In one cat, the somatic branch also was recorded. It differed from the genital in having a faster conduction for the A process, 103 m.p.s., which would indicate the presence of an α wave; a relatively much lower B_2 component, and little if anything in the B_1 position. The C process was present. Comparison of the histological sections of these two branches (fig. 10) shows the distribution that would be expected on the basis of the inferences drawn above.

While the tracing of functional relationships is beyond the scope of this paper, the distribution of the genital nerve suggests the innervation of

smooth muscle, in the cremasteric (or round ligament). Such innervation of smooth muscle in this region might account for the relative prominence of the B_2 elevation in the genital branch of the nerve, as compared to its somatic branch.

3. *Histological.* In figures 7 to 10 are shown histological cross sections of some of the nerves here studied,[4] the physiological properties and potential records of certain of which have been included in the preceding divisions of this paper. It will be apparent that the more complete the potential picture of a given nerve, and the more kinds of fibers, as indicated by experiment, there are to be searched for in the anatomical picture, the more complicated will be the situation, and the less satisfactory and convincing will be any correlation that might suggest itself between function and structure. We have, therefore, sought for simple nerves which had as few components as possible; although in most of the nerves we have studied all but the *A* type of fibers were represented, both anatomically and physiologically. It is not certain just what proportion of fibers of a given type would be necessary to produce a potential we could detect. Neither is it clear just where a line could be drawn between types of fibers which in the extreme condition appear to be very distinct anatomically. It is possible, in spite of appearances to the contrary, that between what we have called two types of fibers, intermediate fibers may exist. Also, other kinds of axons than those recognized may contribute to the observed potentials. What is said here, therefore, is meant to apply to the average fiber, or to those which give a distinctive character to the physiological or histological picture.

Histological evidence has been offered (Heinbecker, 1930a, fig. 8) that in certain turtle cervical sympathetic nerves, the absence of the B_1 group of waves from the potential record may be correlated with the absence of

[4] We wish to thank Doctor Cowdry of the Department of Cytology for suggestions and criticism, and for putting the facilities of his laboratory at our disposal. We are especially indebted to Mr. Masek of that department, not only for making the sections of nerves worked upon, but also for a rather satisfactory counterstain of unmyelinated fibers of both warm- and cold-blooded nerves. Sections three to six mu thick, of osmicated nerves, are stained in thiazene red and aniline oil; the sheathes of the unmyelinated axons, only slightly stained by the osmic, are colored red, the axons themselves remaining clear. The background is lighter pink, with myelinated axon sheaths retaining the black stain of osmic acid. Without this counterstain unmyelinated fibers stain a greenish grey in osmic, with little contrast against the background of interstitial tissue, to the eye. Without the counterstain, however, they often show more clearly in photomicrographs than by direct observation in the microscope, owing to the poor actinic contrast between red and black for the photographic plate.

We are under obligation to Dr. Otto Schwartz and his staff of the Department of Obstetrics for kindness in allowing the photomicrographs to be taken with his equipment.

myelinated fibers larger than 4μ from the cross section. On the basis of this and of the cat and rabbit sympathetic pictures we feel justified in referring to these larger myelinated fibers as the B_1 fibers of this and comparable nerves. In all the nerves of which we have both sections and potential records, the absence of a detectable potential in the B_1 region is accompanied by a relative scarcity of larger fibers, while the converse is equally true for the remaining. Whether the few fibers seen in some sections in whose nerve no B_1 component was observed, are sufficient to give a detectable potential, or whether they really, in spite of their large size, have the character of B_2 fibers, cannot be decided, but the first alternative is inferred to be correct. Refractory period measurements and conduction rates have been correlated frequently enough to assure us that the presence of an A or of a B_1 process of significant amplitude in the potential record can be recognized with certainty, although the B_1 and B_2 potentials in these nerves overlap. In the two turtle *vagus* nerves so far studied in which no B_1 wave appeared, *all* the myelinated axons present were very small, the picture being as striking as in the most extreme cases of sympathetic nerves.

Figs. 8, 9, 10. Histological sections of mammalian nerves. Osmic acid, ×880. The nerves were fixed 1 to 3 hours after removal, during which time potential records had been obtained from them. Since different areas of one cross section often show quite different fiber pictures, a region in each nerve has been selected for photographing that was judged to show the most significant details. These regions do not always show average conditions for the whole nerve, as noted below. *8–1* and *2*. Cat sympathetic and separate depressor nerve of same cat. The faintly recorded masses between the myelinated axons of *1* are filled with unmyelinated axons. Record *2* shows many large or B_1 axons almost absent in record *1*, fewer small myelinated or B_2 axons, and regions of unmyelinated axons, the latter more prominent in this region than in the nerve as a whole.

3 and *4*. Rabbit sympathetic and depressor as above. The sympathetic here shows more unmyelinated axons than the average of the nerve, the depressor is a fair sample.

Fig. 9–*1*, *2*, *3*. Cat, rabbit and human vagus nerves, respectively, showing each three types of fibers at least, the chief area of large myelinated axons responsible for the A group of the cat nerve not being in the picture. The relative preponderance of unmyelinated axons in record *9–1* as compared to *8–1*, sympathetic of the same cat, corresponds to the relative magnitude of the C elevations of potential observed (fig. 7) *4*, branch of coeliac plexus of cat, to show large myelinated B_1 axons, a few small myelinated B_2, and the majority unmyelinated or C. Relative values of potentials and fiber areas discussed in text.

Fig. 10, *1*, *2*. Dorsal skin and lateral genital branches of the genito-femoral of a cat. Discussed in text. *3*, genital branch of same nerve, human, A, B_1 and B_2 components were recorded. Many unmyelinated axons are faintly visible. Potential record in figure 7, record *10*. *4*, 4th lumbar grey ramus of dog which gave a small B_1 potential, a larger B_2, and a medium C elevation. Many unmyelinated axons, present under the microscope but not noticeable in this photograph, lie scattered between the small myelinated axons.

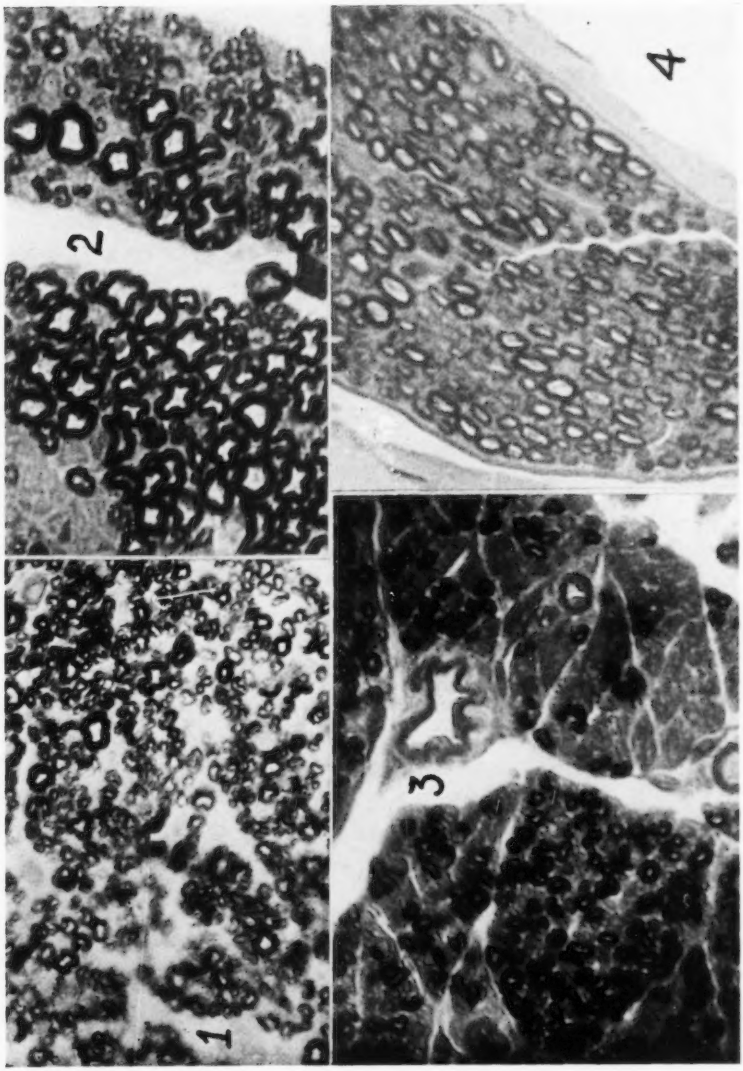

Fig. 8

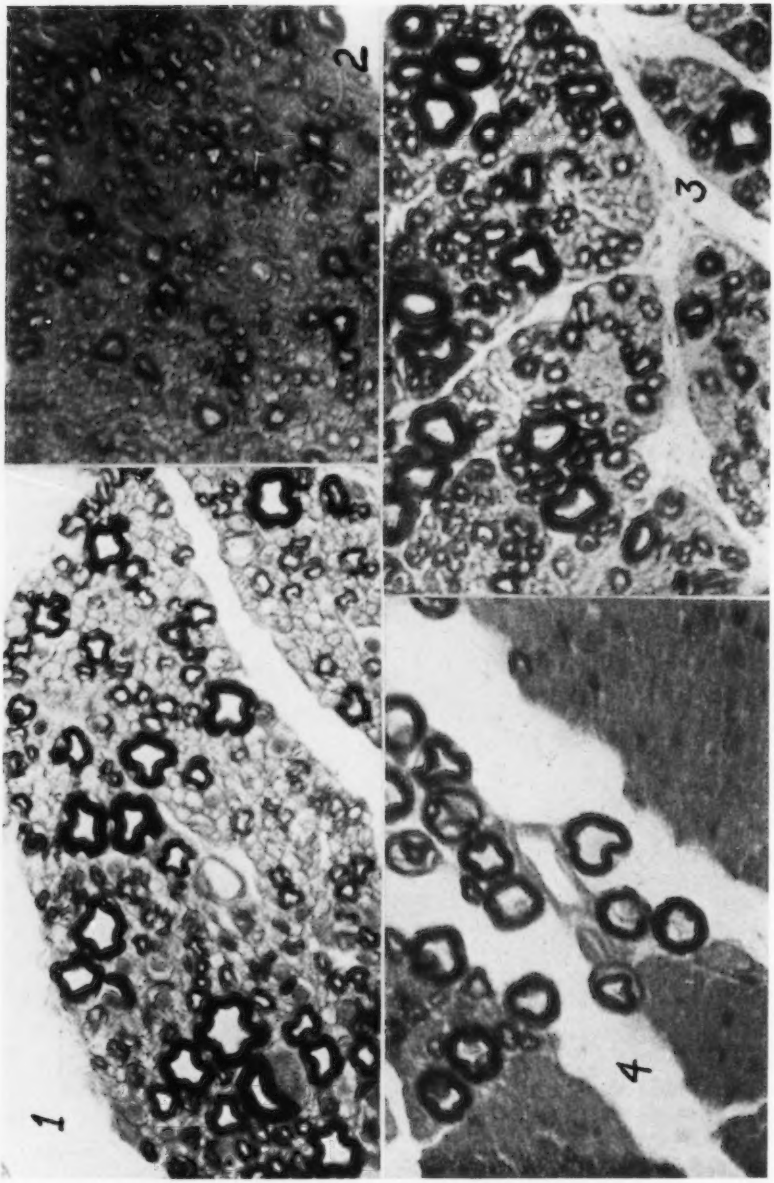

Fig. 9

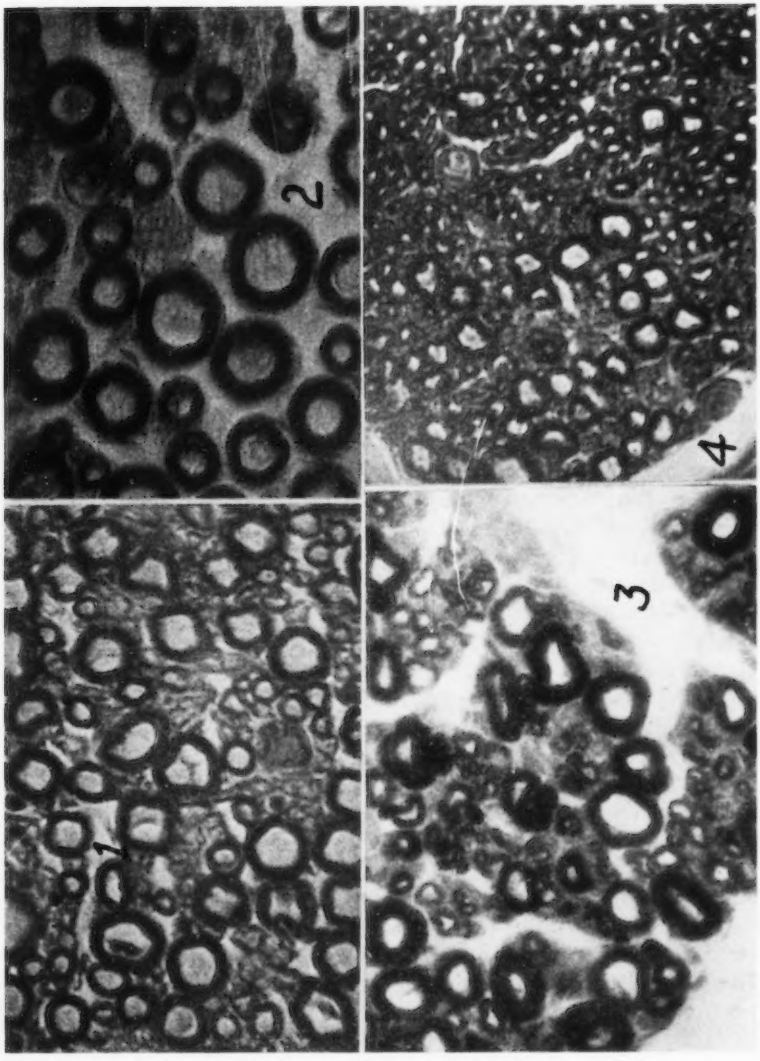

Fig. 10

Section 1 and 2, figure 8 are from the sympathetic and depressor nerves of the cat previously discussed which had a separate depressor nerve (fig. 7, records 6, 7, 8). The earliest potential recorded from the sympathetic was a B_2 process, with a refractory phase of 4.5 sigma and a rate of 14 m.p.s. The depressor on the other hand gave a large B_1 process, 32 m.p.s., refractory period 0.75σ, a small late B_2 10 m.p.s. and $5 + \sigma$, and a C. Again, as in the turtle, fibers of large size are relatively absent from the sympathetic and present in the depressor; while small thinly myelinated and unmyelinated are present to account for the B_2 and C elevations in both nerves.

Exactly comparable results were obtained from a rabbit sympathetic and depressor recorded and sectioned for comparison (sections 3 and 4).

In all the mammalian vagus nerves studied, (fig. 9, records 1 to 3) the results were similar; an A elevation was present, the B_1 was relatively lower than in the accompanying sympathetic, the B_2 was no higher, or more usually (as in the cat vagus records of figure 7) was less prominent than in the sympathetic, and the C elevation was very large. For comparable components, other properties investigated were like those of the sympathetic. The fiber group distribution in these nerves corresponds in general with the magnitude of the potentials assigned to them, the large myelinated and the unmyelinated axons being more numerous than in the sympathetic nerves.

A satisfactory nerve for the differentiation between the fibers giving the B and C potentials was found in a branch of the coeliac plexus of the cat, that ran toward the intestine and spleen. A small B_1 wave was present, comprising about 7 per cent of the total potential area. A B_2 elevation was just detectable but not over 2 to 3 per cent of the total potential; the C wave composed 92 per cent of the total (fig. 7, 11 to 14). Certain larger myelinated axons occur in this section (fig. 9, section 4) that account for the small B_1 component, and the scattering small myelinated fibers would, on the basis of a comparison with the previous records, be expected to give a B_2. Either there are not enough of these to give more than a barely detectable potential, or else some of them at least must be small B_1 fibers, which would then lie in the B_2 size range, although differing in their other properties. The striking fact is the relationship between a preponderance of unmyelinated axons, and the preponderance of the C wave, which in this case is certainly assignable to these fibers.

An estimate was made of the area occupied by the fibers inferred to be responsible for the B_1 component of potential; 120 occurred in a section, 8 to 10μ in diameter.[5] The sum of the areas of the sections of these fibers

[5] This B_1 component conducted in this nerve 25 m.p.s. The cat vagus A wave with a fiber size of 15 μ maximum, conducts up to 80 m.p.s. If the large fibers of this visceral nerve were A fibers, the rate to be expected on the basis of Gasser and

was just over 10 per cent of the total area of the nerve section. On the basis of our inference, 10 per cent of the nerve's material then gave 7 per cent of the total potential, and something under 90 per cent gave 92 per cent of the total response, a correspondence as good as can be expected when we know nothing about the relative potential areas per fiber of such nerves.

This correspondence is supported by comparison with the *B* process of the sympathetic referred to previously, where the B_2 component alone present is quite as certainly assignable to the multitude of small myelinated axons; since the relatively large *C* wave from the sympathetic nerve must be attributed, on the basis of the visceral nerve findings, to the numerous unmyelinated axons present in the sympathetic.

A grey ramus of the dog from the lumbar region (fig. 10, section 4), which gave a small B_1 and a medium B_2 potential, shows a content of small myelinated axons which is between that of the sympathetic nerve and that of the visceral nerve discussed.

Sections of the genital branch of the genito-femoral from the cat and human (fig. 10, sections 1 and 3) have closely similar fiber contents, and the potentials (fig. 7, records 9 and 10) are equally similar. Experiments on the rabbit and dog gave the same results. The human nerve gave no *C* wave, presumably due to the time of the record after removal of the nerve, although on the basis of more recent experiments it is possible that, especially if it was depressed, stimuli of longer duration than those employed would have been effective. Attention may be called to the large thickly myelinated axons in these sections interspersed among the others less heavily myelinated; comparison with other nerves suggests that the more heavily myelinated axons are somatic, but whether motor or sensory has not been determined. The larger fibers that are more thinly myelinated then presumably include afferents passing through the sympathetic system, similar to the large fibers in other sympathetic nerves.

In these specimens of genito-femoral, there is a tendency for the axons to be collected in distinct islands or segments separated by trabeculae, a condition which is characteristic of the involuntary nerves studied in both turtle and cat, but which does not appear so prominently in, for instance, the cat sympathetic and vagus. In the human vagus (fig. 9, record 3) such segmentation is again prominent, especially in regions where small fibers

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Erlanger's (1926) work on *A* fibers would be close to 50 m.p.s. While the B_1 component is in our experience the most easily depressed of any present, this rate seems too slow to correspond, even allowing for such depression as might have occurred. Moreover, the corresponding B_1 component in the turtle sympathetic and vagus nerves, when present, has a rate not much over one half as fast as the size of the fibers would suggest; these rates presumably correspond to that of the *B* element in the dog saphenous reported by Erlanger (1927) but are still almost twice as fast as the B_2 component reported by Heinbecker (1929).

predominate, and the rabbit sympathetic and vagus both show it. In the cat sympathetic lacking the B_1 component, close inspection under the microscope reveals the same condition, which in other cat sympathetic nerves is obscured by the presence of the many myelinated fibers; as illustrated also, in the cat depressor as compared to its accompanying sympathetic trunk. As the proportion of large fibers increases in general, these islands are broken up into smaller areas, but can still be recognized in the microscope. The appearance seems to be due to the staining of the sheaths of the unmyelinated axons a greyish green by osmic acid, with a similar staining of the matrix of connective tissue in their interstices. Thiazene red counterstaining emphasizes these areas by staining both unmyelinated axon sheaths and connective tissue, the latter pink and the former a deeper red, while the axon cores remain unstained. Such a matrix material does not occupy the interstices between the larger myelinated axons, when unmyelinated axons are not also present in the vicinity. When all types of axons are present, the A and B_1 fibers are scattered either throughout these areas or between them, while the B_2 axons appear to be more closely associated in general with unmyelinated fibers.

Discussion. The average values for the time functions of the nerves of warm- and cold-blooded animals are in general comparable, if we allow for differences in temperature, but the temperature coefficients are not demonstrably those that have been found to fit the conduction rate of myelinated nerves (Lillie, 1923; Gasser and Erlanger, 1927). For instance, the refractory periods of turtle and cat A and B_1 components are 0.8 and 0.6σ, at 25 and 37°C., respectively; of the B_2, 3.5 and 2.5σ, of the C 5–7 and $4–5\sigma$. There are the greatest variations in the data obtained from the slowest axons, which are also easiest fatigued; the deviation may be partly due to this, and partly to the technical difficulty of recognizing a second response of this type, when it falls close to the first. Conduction rates of the slower fibers have temperature coefficients comparable to those of the faster.

The data presented in this paper permit of two general inferences, the validity of neither of which depends upon the correctness of the other. First, the properties of typically somatic large myelinated fibers have time values of a different order of magnitude from those of the typically autonomic fibers. This is consistent with such findings as those of Lapicque and his co-workers (1926), that the chronaxie of a nerve fiber tends to correspond with that of the organ innervated; although we have not investigated the precise degree of this correspondence between nerve and organ. The larger myelinated afferents running with autonomic fibers have properties closely similar to somatic fibers, although they presumably come from viscera innervated by autonomics. Other afferents, i.e., small myelinated or unmyelinated afferents, if any occur in these nerves, possibly have

properties similar to those of the autonomic efferents. We have not made functional tests to detect such afferents.

The second inference which seems to us permissible is that the B_2 component arises from small myelinated axons, the C from unmyelinated. The almost universal presence of both types of axons as observed physiologically and histologically in the same nerve renders this conclusion insecure at present, but we have found no evidence against it, and the correspondence between the relative numbers of axons of the two types and relative area of B_2 and C potentials is in general satisfactory. We wish, however, to limit this inference for the present to the condition in nerves in which the correspondence is unmistakable, anticipating, of course, that the finding will prove to be general.

With regard to the first statement, the distinction between somatic and autonomic fibers on the basis of the properties here employed for their differentiation, might be called into question by Erlanger and Gasser's (1930) finding that the B elevation of the saphenous of the dog and cat enters the sciatic trunk from the rami, and is not present in the last lumbar and first sacral sciatic roots, and by their further inference that the fibers giving rise to it are postganglionic. These B fibers, they find, have a short duration of axon potential like somatic fibers, as contrasted to the longer duration reported by us (1929) for the B_2 component of autonomic nerves. They also find a faster rate for the sciatic and saphenous B than we find for the autonomic B_2 efferent. We have applied the criterion of refractory period duration to the saphenous B wave, and find it virtually the same as that of the somatic A and the autonomic nerve B_1 component (0.6 to 0.7 sigma) as contrasted with a duration of about four times this value for the autonomic efferent B_2. There was no detectable element of this saphenous wave with B_2 properties. These findings all indicate that the cat and dog saphenous wave arises from axons of the type which in autonomic nerves convey visceral *afferent* impulses.

In correspondence with this suggestion, Kuntz and Farnsworth (1928) report on the basis of degenerative experiments that medium sized and small myelinated axons pass through the dorsal roots and white rami in the lower thoracic and upper lumbar region, course downward through the sympathetic trunks and enter the somatic system through the lower lumbar and upper sacral grey rami. These axons are histologically of the type usually assigned an afferent function. This work, therefore, and Erlanger and Gasser's finding that the B component of the sciatic-saphenous trunk enters through the grey rami, together with the correspondence between the properties of these axons and others in the autonomic system known to be afferent, permit of the inference, tentatively at least, that the B elevation of the dog saphenous is not post-ganglionic but afferent, and contributes to these somatic nerves what we have called in the autonomic nerves the B_1 *component.*

Our second general inference (in a preliminary report, 1929) has been specifically called into question by Erlanger and Gasser (1. c.) on the basis of their finding that the frog motor 7th roots send into the rami only a double *C* wave, whereas the rami were . . . . "made up almost exclusively of thinly sheathed myelinated fibers" . . . . In experiments where the nerve distal to the ganglion was stimulated, a lead from the ramus gave a double *C* wave but no *B*. In repeating Erlanger and Gasser's experiment stimulation of the 7th nerve distal to the ganglion gave a *B* wave in the ramus of our preparation travelling 1.8 m.p.s. and a *C* wave 0.4 m.p.s. of about equal height, followed by a lower second *C* wave. We cannot reconcile these apparently conflicting results. In sections of several 6th and 7th nerve rami of the frog we have observed from one-third to one-half the axons to be unmyelinated. The grey and white rami of the frog when sectioned through the region where they lie together are indistinguishable in relative content of myelinated and unmyelinated axons, and these authors report a *B* and a *C* wave contributed to the sciatic by the grey rami.

In this connection the previously known fact, which we have checked in two 6th nerve preparations, that the white ramus and nerve contain many more fibers than the roots, suggests a possible explanation for the *B* wave not being transmitted between root and ramus. In one of the nerves studied, owing to the fact that the ramus could be followed up the trunk as a separate bundle, it could be demonstrated by fiber counts that many of the ramus fibers ended at the dorsal root ganglion level, and did not enter the motor root. At any rate the root *C* elevation which passes into the ramus will not "completely account for" the white ramus potential, as Erlanger and Gasser suggest it will, until the white ramus fibers which do not enter the motor root have been accounted for.

We have met with no evidence in this work that unmyelinated afferents were present, but the possibility is not excluded. These might be particularly expected in the depressor, which is generally considered to be only afferent. If they occur here in sufficient numbers to be detectable (and a well defined *C* potential is present), they appear to have approximately the same refractory phase and conduction rate as efferent *C* fibers. The same would apply to the B_2 group of this nerve if it were shown to arise from afferent fibers. It may be emphasized here that the *smaller* of the myelinated B_1 fibers and the small myelinated fibers presumed to give rise to the B_2 component cannot be distinguished at present by purely anatomical means, there being no size differentiation possible. The solution therefore waits upon experiments conducted in the body, in which reflex effects would be looked for in response to stimulation at a strength known to be demanded for excitation of the B_2 potential.

It is most interesting to note that the duration of the electrical process

in unmyelinated axons corresponds to the high rate of metabolism of activity reported by Meyerhof (1929) for cold-blooded unmyelinated nerves. Meyerhof's values for crab nerve were 10 to 20 times those of Gerard and Meyerhof (1927) for frog sciatic, a ratio comparable to the times-to-maximum, or duration, of the potentials from the fastest and slowest fibers of mixed nerves. It is not to be concluded, however, that the potentials of these different axons are direct measures of the underlying chain of chemical processes that constitute excitation or response, to say nothing of recovery; for the refractory periods, which one would naturally infer to be measures of some phase of that activity, vary with fatigue and other conditions quite independently of the duration of the potential, so far as has been detected to date. Conditions obtaining during the relatively refractory or recovery period have not been investigated here, except for measurements in some instances of return of irritability after the refractory phase.

If such a correspondence between physiological properties and histological aspect should prove to hold in general for involuntary nerves, it obviously becomes possible to predict, from any convenient criterion, the rest of the attributes of the fibers concerned.[6] Histological cross sections made after an experiment is over, of small or short branches going to individual organs or parts of organs for instance, whose action potentials might be recorded with difficulty, especially *in situ*, might then give fairly complete information about the axons concerned in stimulating the organ to activity. Taken together with the knowledge of relative thresholds for the different axons in a given nerve, it should be possible to conduct experiments on animals by stimulating nerves at known thresholds, and examining afterwards the nerve cross section, without complicating the procedure with the technical vicissitudes sometimes attendant upon recording action potentials during *in vivo* experiments.

SUMMARY

1. By means of the cathode ray oscillograph technique, four components of potential can be recognized in certain autonomic nerves. The vagus and the genital branch of the genito-crural may be taken as types containing all four components. Other nerves lack certain of these elements. The potentials are termed for convenience the A, B_1, B_2 and C components.

2. Determinations have been made of the properties characteristic of the fiber groups which contribute to these potentials. The first two groups

[6] The presence of myelinated fibers with a C rate, as reported by Erlanger and Gasser, and of unmyelinated afferents or of afferents of the B_2 type in a nerve would complicate this procedure. Such axons have not been detected by us so far in the nerves we have studied.

are similar to each other in certain respects, the last two likewise resemble each other, the major differences in certain properties occurring between the second and third (B_1 and B_2) components.

3. The properties which change more abruptly from the B_1 group to the B_2 are threshold, duration of axon potential response, chronaxie and absolutely refractory period, indicating a lower irritability and a slower activity and recovery in the last two groups, as compared to the first two, in the ratio of at least four to one.

4. Histological cross sections of nerves from which potentials have been obtained permit of inferences as to the anatomical character of the type of fiber contributing to each potential. The first or A potential arises from large myelinated fibers of the type supplying skeletal muscle and peripheral afferent endings, i.e., somatic, motor and sensory. The B_1 potential arises from fibers of the type usually assigned to visceral afferent function, passing through autonomic nerves. The B_2 and C potentials arise from fibers which in certain nerves can be identified as autonomic efferents. Of these two groups, the B_2 is inferred to contain the small thinly myelinated axons present in the autonomic system, the C the unmyelinated.

5. The critical findings upon which the above inferences are based are obtained from nerves from which certain potential components are small, or are not obtained, and in whose cross sections certain types of axons are correspondingly few in number or not present; in such a case the missing potential is inferred to correspond to the missing axon type. Whether the B_2 and C groups of axons are exclusively efferent has not been investigated.

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THE HEART RATE OF UNANESTHETIZED NORMAL, VAGOTOMIZED, AND SYMPATHECTOMIZED CATS AS AFFECTED BY ATROPINE AND ERGOTOXINE

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Recently in the Harvard Physiological Laboratory the denervated heart has been employed as an indicator of chemical changes in the blood (see Cannon and Britton, 1927; Moore, 1929). The denervation was accomplished by removal of the upper thoracic sympathetic chains, section of the right vagus nerve below the recurrent laryngeal branch, section of the common cardiac nerve, and severance of the cardiac branches of the left vagus, along with bilateral abdominal sympathectomy and splanchnotomy to abolish reflex secretion of adrenin (see Cannon, Lewis and Britton, 1926). In certain instances some vagus fibers were inadvertently left uncut and atropine was then employed to see what might be the effects of chemical disconnection of the heart from the central vagus mechanism. In other instances of complete vagal disconnection of the heart, emotional excitement resulted in a slow rise in heart rate which required minutes to reach its peak, and this effect failed to occur after removal of the remnant of the sympathetic chains. The result just mentioned led to the use of ergotoxine to learn whether the slow rise could be eliminated. During the course of these observations on the action of atropine and erogtoxine, some effects were noted which made it seem worth while to undertake a series of tests of the action of these drugs upon the heart rate after complete exclusion of sympathetic impulses throughout the entire body (method described by Cannon, Newton, Bright, Menkin and Moore, 1929), and after exclusion of vagal effects on the heart. The necessary operations were performed with aseptic technique. Since the animals completely recovered and remained healthy and vigorous for long periods thereafter, the tests which were made are valuable as showing the changes in the heart rate brought about by sympathectomy and by vagotomy, together with the effects of atropine and ergotoxine, in a simple and direct manner without the disturbance of anesthesia.

Observations on normal animals. As a basis for later comparison of the

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effects on animals which had been operated upon, observations were first made on the heart rate of normal cats and the action of atropine and ergotoxine upon it. In order to obtain a basal rate the cat was placed upon a cushion supported by the lap of the observer and was allowed to rest there quietly until the heart rate fell to a low level at which it continued. This condition of being quiet on the lap is referred to in the tables as "QOL". The determinations were repeated day by day until the animal had become thoroughly tamed and accustomed to the procedure and it was then that the lowest readings were obtained. The figures in the first section of table 1 show the heart rates which were either counted by means of the stethoscope or were actually recorded graphically. The basal rate in certain instances was surprisingly low. To show that such rates are actual, however, the record of a low rate is reproduced in figure 1. After the basal

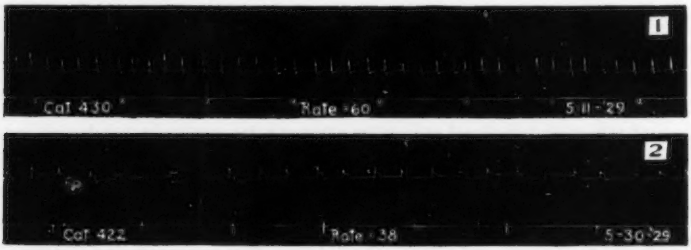

Fig. 1. Original record of cat 430, showing the basal heart rate of 60 beats per minute associated with marked respiratory arrhythmia. Time in 5-second intervals.

Fig. 2. Original record of cat 422, after sympathectomy but with vagi intact, showing the extremely slow pulse of 38 beats per minute when under the influence of ergotoxine. Time in 5-second intervals.

rate had thus been observed or registered the animal was fastened back-downward on a holder where it was comfortable but restrained. In this position it became emotionally excited and struggled quite vigorously. The result of the struggle was a great increase in the heart rate. As shown in table 1 the average rate per minute rose from 97 to 237 beats—an increase of 140 beats or 144 per cent. The maximal rate in one instance reached 312 beats per minute.

The increase of heart rate resulting from struggle may be the effect of a variety of circumstances. There is release from vagal inhibition, there is increase of direct sympathetic stimulation of the heart, and there is release into the blood stream of extra adrenin which collaborates with the sympathetic impulses in the operation of the sympathico-adrenal system. The problem that is of interest is the rôle which these various agencies play and the effect of atropine and ergotoxine upon them.

The effects of atropine. As shown in table 1 the effect of atropine in the normal cat (1 mgm. per k. to the fasting animal), when observed about 30 minutes after its administration, was an increase of well over 100 per cent in the basal heart rate, which rose from an average of 97 beats per minute to an average of 208 beats per minute. At the same time the average maximal rate that followed struggle was raised under atropine from 237 beats per minute to 272.

TABLE 1

Heart rates of normal cats (18 to 20 hours after last feeding), when quiet on lap (QOL) and after struggle; also effect of atropine (1 mgm. per kilogram) after cardiac vagotomy or after total exclusion of sympathetic impulses. Respiratory arrhythmia (RA) is recorded as slight or moderate (+), or as marked (++) or as absent (0)

CAT NUMBER	CAT NORMAL—NO OPERATIONS						AFTER VAGOTOMY—SYMP. INTACT						AFTER SYMPATHECTOMY—VAGI INTACT					
	Before atropine			After atropine			Before atropine			After atropine			Before atropine			After atropine		
	QOL	Strug.	RA	QOL	Strug.	RA	QOL	Strug.	RA	QOL	Strug.	RA	QOL	Strug.	RA	QOL	Strug.	RA
413	106*	240	+	237	300	0												
414	73	135	++	195	232	0	114	168	0	154	196	0						
415	72	140			224	0	122	196	0	150	196	0						
416	78	200	++	190	270	0	106	240	0	155	252	0						
417	104	260	+	225	300								59	132	+	151	142	0
422	96	288	++										71	120	++	158	160	0
423	122	300	+	176	292	0							76	176	++	160	180	0
424	128*	296	+	204	302	0							96	172	++			
425	94	240	+	187	272	0												
426	115	210	+	263	288	0												
427	98	300	++				139	264	0	146	252	0						
428	94	240	++	200	252	0	157	240	0	166	240	0						
429	114*	312	+	240	280	0												
430	60	172	++	180	252	0												
Average	97	237		208	272		128	222		154	227		76	150		156	161	

* Animal not satisfactorily quiet.

Another effect which atropine had on normal cats was that of abolishing respiratory arrhythmia ("RA"). This phenomenon, as shown in figure 1, was a regular accompaniment of the basal heart rate, and characteristically was more marked in animals with a very low basal rate than in the others. After the administration of atropine the phenomenon was consistently absent.

As shown in the second division of table 1, the surgical interruption of the vagal paths to the heart raised the average basal heart rate from 97 beats

per minute to 128 beats, a rise of 31 beats or of 32 per cent. Roughly this difference represents the tonic inhibitory effect which the vagus exerts on the rate of the heart.

Associated with vagotomy there was a total absence of respiratory arrhythmia, quite analogous to that observed after the chemical stoppage of vagus impulses by atropine in the unoperated cats. The absence of respiratory arrhythmia in these animals indicated that vagotomy had been complete.

When atropine was given to the vagotomized cats there was a further increase of the heart rate from an average of 128 to an average of 154 beats per minute. Since the vagi were already severed the effect of atropine could not be explained by a blocking of vagal impulses. The increase of rate, therefore, would seem to be due to the stimulating action which atropine has on the central nervous system. The animals were definitely excited as an effect of the drug. This inference is in harmony with the effects of atropine on normal animals. As shown in the first division of table 1 the average heart rate after atropine was not in the neighborhood of 128—which might be expected if atropine, like vagotomy, merely disconnected the heart from vagal influence—but rose to 208 beats per minute. That atropine can cause a greater cardio-acceleration than can be explained by its paralysis of parasympathetic endings is not a new finding. Dresbach and Waddell (1926) reported a marked cardio-acceleration after atropine in a vagotomized cat and Rogers (1920) reported a tremendous rise of heart rate following atropine in two vagotomized dogs in which electrical stimulation of the vagus failed to produce any slowing of the heart. The experiments reported here, however, serve to show not only that the central stimulation of the cardio-accelerator mechanism by atropine is a usual effect in the unanesthetized animal, but also that this effect may be far greater than that produced by paralysis of the parasympathetic.

Struggle caused a considerably faster beat after vagotomy whether atropine was active or not. The average maximal figures, as shown in the second division of table 1, were approximately the same in the two conditions.

Another interesting observation shown in table 1 was the discrepancy between the heart rates after the administraton of atropine in the normal cat—a basal of 208 rising to 272 on struggle—and those following atropine after the same cats had been vagotomized—a basal of 154 elevated by struggle to 227. In no instance was this discrepancy lacking. A reasonable basis for its explanation is found in the arrangement of the cardiac nerves. Many sympathetic cardio-accelerator fibers enter the vagus trunks from the inferior cervical and the stellate ganglia. Furthermore, some of these sympathetic fibers unite with parasympathetic fibers to

form the common cardiac nerve. Thus section of the right vagus trunk, the common cardiac nerve and the cardiac branches of the left vagus is unavoidably associated with interruption of the major bundles of accelerator fibers. There still remain cardio-accelerator fibers from the upper thoracic sympathetic chain (see Cannon, Lewis and Britton, 1926). By its central effect, therefore, atropine still causes the heart to beat faster, but the maximum effect is lacking because most of the peripheral accelerator connections have been cut. The appreciable loss of cardio-accelerator effect, thus accounted for, directs attention to a fact which may be overlooked. The gross visceral nerve which supplies a thoracic or abdominal organ is often a mixed nerve, not only in containing both visceral afferent and autonomic fibers, but also by including among the latter group both sympathetic and parasympathetic elements.

After sympathectomy, with the vagi still intact, as shown in the third section of table 1, the average basal heart rate fell to 76 beats per minute. This drop of approximately 20 beats per minute from basal rate of the cats before operation may be interpreted as a rough measure of the action of tonic vagus impulses in the absence of opposing sympathetic impulses. Associated with this low rate was a marked respiratory arrhythmia. The striking feature of the figures in the third section of table 1 is that struggle before or after atropine and the quiet state after atropine were associated with approximately the same rate of the heart. This is understandable in the absence of sympathetic and adrenal influences. In the absence of these influences the acceleration of the heart would be dependent wholly on inhibition of vagal tone or blockage of vagal impulses. If vagal impulses were wholly blocked by atropine, struggle would not produce a great change. The figures actually observed tend to support this interpretation.

The effects of ergotoxine. In table 2 are presented the results of obsertions and records of the heart beat of cats under the same conditions as those which prevailed in the observations on the action of atropine. With a few exceptions these were indeed the same animals that are reported on in table 1.

As shown in the first division of table 2, ergotoxine (Burroughs-Welcome), when given to normal animals, causes a decrease in the basal heart rate. Doses of 1 mgm. per k. to fasting cats, with observations usually made 20 to 30 minutes after the injection, caused the average basal rate to drop in these animals from 94 beats per minute to 80. Associated with this effect was a very great reduction in the maximal rate caused by struggle—a reduction from 240 to 169 beats per minute.

In striking contrast to the figures just cited are those which are seen in the second division of table 2 when ergotoxine was given to the vagotomized cats. After vagotomy the average heart rate was 128 beats per

minute. Ergotoxine caused a marked drop in the basal rate in only one (cat 415) of five animals whose cardiac vagal control had been eliminated. It would appear therefore that one of the effects of ergotoxine is to cause a stimulation of the vagal centers and thereby a slowing of the heart. In the absence of vagal connections this effect largely disappears. The rise of heart rate after struggle in spite of ergotoxine, as seen in cats 415 and 427, seemed a rather surprising phenomenon, for the drug is supposed to paralyze sympathtic endings and these were the only endings present and affect-

TABLE 2

Heart rates of normal cats (18 to 20 hours after last feeding), when quiet on lap (QOL) and after struggle; also effect of ergotoxine (1 mgm. per kilogram to fasting cat) after cardiac vagotomy or after total exclusion of sympathetic impulses

CAT NUMBER	CAT NORMAL—NO OPERATIONS				AFTER VAGOTOMY—SYMP. INTACT				AFTER SYMPATHECTOMY—VAGI INTACT			
	Before ergotoxine		After ergotoxine		Before ergotoxine		After ergotoxine		Before ergotoxine		After ergotoxine	
	QOL	Strug.	QOL	Strug.	QOL	Strug.	QOL	Strug.	QOL	Strug.	QOL	Strug.
414	73	135			114	168	118					
415	72	140	90		122	196	104	160				
416	78	200	80	160	106	240	140					
417	104	260	91	150					59	132	48	84
422	96	288	68	180					71	120	38	65
423	122	300	79	180					76	176	60	120
424	128*	296	84	180					96	172	58	
425	94	240	88	168								
427	98	300	80	170	139	264	135	160				
428	94	240			157	240	144					
429	114*	312	92									
430	60	172	51									
Average	94	240	80	169	128	222	128	160	76	150	51	90

* Animal not satisfactorily quiet.

ing the heart. The point has been made, however, (Dale, 1906) that ergotoxine is less effective in blocking sympathetic impulses to the heart than to other structures, or may be quite without influence (Otto, 1927). It is noteworthy that the rate after struggle was not so high as it was in the absence of ergotoxine; in this respect the vagotomized animals were like the normal animals under ergotoxine.

The foregoing inference regarding the effect of ergotoxine on vagal tone is reinforced by the results reported in the third division of table 2. The total removal of sympathetic impulses leaves only the vagus in action, and the heart is thus made to beat very slowly. After ergotoxine, however,

this slow rate is reduced still further and in one instance the almost incredibly low rate of 38 beats per minute was recorded (see fig. 2). The effect of a struggle was to raise the average basal rate from 51 beats to 90. When cat 422 (fig. 2) was excited the rate rose from 38 to 65 beats per minute, and then *gradually* fell to 40, indicating quite clearly that the slow rate was not due to heart block. It is of some interest to note that in three of the animals whose heart rates were greatly reduced by the action of ergotoxine after sympathectomy the increase of rate after struggle was approximately of the same percentage.

From the foregoing observations it is clear that in the unanesthetized animal ergotoxine in a dose of 1 mgm. per k. has a central action which increases vagal tone and that such a dose may not greatly check the effects of sympathetic influences on the heart.

SUMMARY

This paper reports observations on the heart rate of unanesthetized cats which were either normal or had recovered from vagotomy or sympathectomy.

The average minimal heart rate of normal, resting, unanesthetized cats is about 95 beats per minute and is associated with respiratory arrhythmia (see fig. 1), which is more marked the slower the rate. The respiratory arrhythmia is abolished by atropine or by vagotomy (see table 1), but not by sympathectomy. The average heart rate rises to about 240 beats per minute after struggle (see tables 1 and 2).

In the normal cat atropine raises the average resting or basal rate to 208, and the rate after struggle to 272 beats per minute (table 1). In the same animals erogotoxine lowers the basal rate to 80, and the rate after struggle to 169 beats per minute (table 2).

After *vagotomy* (in which some cardio-accelerator fibers are cut) the basal heart rate is increased to about 125 beats per minute. Atropine still further raises the average basal rate (to about 155), but it does not change to a noteworthy degree the average rate produced by struggle (see table 1). In this preparation ergotoxine does not markedly influence the basal heart rate; the rise due to struggle is, as a rule, reduced (see table 2).

After *sympathectomy*, with vagal supply to the heart left intact, the average basal rate is reduced to about 75 beats per minute. Atropine raises this basal rate to about 155, and struggle raises it little further (to about 160). In this preparation ergotoxine greatly reduces the average basal rate (to about 50 beats per minute) and also reduces the effects of struggle (to about 90 beats per minute).

The inference is drawn that atropine and ergotoxine have not only peripheral effects, but that in the unanesthetized animal atropine stimulates

centrally cardio-acceleration and ergotoxine stimulates centrally cardio-inhibition.

After sympathectomy the vagal mechanism can slow the heart of the unanesthetized cat from a maximum rate of about 150 beats per minute to a minimum of about 75 beats per minute, whereas after vagotomy the sympathetic mechanism can speed up the heart from a minimum rate of about 125 beats per minute to a maximum of about 225 beats per minute. The respective ranges of operation of the two systems emphasize the view that the vagal mechanism serves especially to assure opportunity for rest and recuperation of the heart, and that the sympathetic mechanism, on the contrary, acts in emergency states when physical effort and stress require the heart to work vigorously.

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THE PHYSIOLOGIC ACTION OF THE VENOM OF THE HONEYBEE (APIS MELLIFERA)

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In previous communications we have reported the results of our studies on the physiologic properties of the venom of the rattlesnake (*Crotalus horridus*). Since we found that the intradermal injection of the dilute venom of rattlesnakes is followed by a reaction comparable to that of the sting of a honeybee (*Apis mellifera*), it seemed probable that the venom of the bee would elicit many responses in animals similar to those produced by the venom of rattlesnakes. Considerable force was given to this conception by the fact that several fatalities are on record as a consequence of the sting of bees. The similarity between the two venoms is the basis of the present report.

As might be expected, literature is extensive on the development and morphology of the stinging apparatus and associated glands of the honeybee. The literature dealing with the chemical nature of the venom of the honeybee is excellent but not voluminous, and reports on the physiologic action of this venom are relatively few. Phisalix (1922) in her book on venomous animals and venoms has given an adequate review of the literature on these subjects. The reader is also referred to a review by Faust (1924).

METHODS. The venom for these studies was collected during the winter months. The cold weather had rendered the bees sufficiently lethargic so that large numbers could be readily removed from the hive with forceps into a container. A few drops of chloroform were thrown into the container. The anesthetized bees were seized by the abdomen and the sting which was thus made to protrude was grasped with a pair of fine-pointed forceps and pulled out. The venom sac almost invariably accompanied the sting. With the aid of a dissecting microscope and a pair of needles the sac was torn open and the venom allowed to diffuse into a mixture consisting of equal parts of Ringer-Locke's solution and glycerine. The dilution was made so that 1 cc. contained the venom from ten honeybees.

EXPERIMENTAL OBSERVATIONS. The results may be presented under three headings: 1, the effect following the intravenous and intradermal

injection of the venom of the bee; 2, the effect on involuntary muscle as exemplified by the bronchioles of the guinea pig, the perfused uterus of the virgin guinea pig, and the perfused heart of the rabbit, and 3, the effect of the venom on dog's blood in vivo and in vitro.

To an etherized dog weighing 4.5 kgm. with a normal blood pressure of 100 mm. of mercury the venom from six bees was given intravenously. After a latent period of twelve seconds there was a sharp rise of 25 mm. followed by a rapid and profound fall, so that within two minutes the blood pressure had descended to 30 mm. (fig. 1).

To determine if this effect was manifested by herbivora, an etherized rabbit weighing 2.6 kgm. with a normal blood pressure of 100 mm. was given an injection of the venom from four bees. After a latent period of ten seconds the blood pressure fell quickly to about 60 mm. At this point it began to rise and within four minutes the normal pressure had been regained. A second intravenous injection of twice the first dose did not produce any further effect on the blood pressure (fig. 2).

Fig. 1. Effect of the venom of honeybees on the blood pressure of a small etherized dog. A preliminary rise in blood pressure was followed by a prompt fall to about 30 mm. of mercury. The animal succumbed quickly. Such preliminary rise is not usual. In this tracing and subsequent tracings, the time marker recorded every five seconds. The base line indicted zero millimeters of mercury.

Fig. 2. The effect of the venom of the honeybee on the carotid blood pressure of an etherized rabbit. At *A* and *B*, the rabbit received, intravenously, the venom from four and eight bees respectively. The second injection is shown to be ineffective.

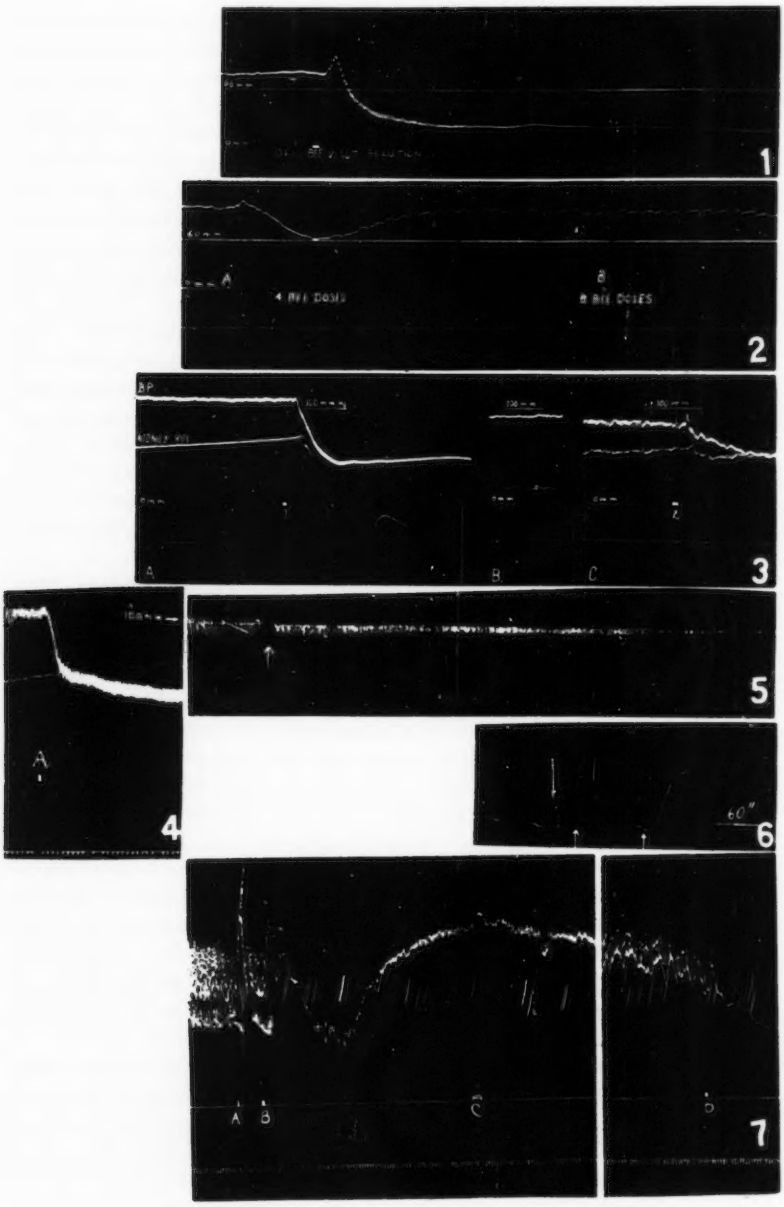
Fig. 3. Effect of the venom of the honeybee on the blood pressure and volume of the kidney of a small etherized dog. The time interval between tracings *A* and *B* and between *B* and *C* is twenty-five minutes in each case. At *1* the venom from ten bees was given intravenously. The injection was repeated at *2*.

Fig. 4. The effect of the venom of the honeybee on the blood pressure and volume of the hind limb of an etherized dog. At *A* the venom from forty bees was given intravenously.

Fig. 5. Ventricular contractions of the isolated heart of the rabbit perfused by a modification of the method of Locke and Rosenheim. At the arrow the venom from eight bees was added to the 200 cc. of perfusing fluid.

Fig. 6. Response of the perfused uterus of the virgin guinea pig to the venom of the honeybee. At the first arrow the venom from one bee was added to the 40 cc. of perfusing fluid. The magnification of the writing lever was 1.5:1. With repeated washing the organ relaxed. The second arrow indicates a repetition of the experiment.

Fig. 7. Pulmonary excursions of a curarized guinea pig receiving, under local anesthesia, rhythmic intratracheal jets of oxygen about forty times a minute. At *A* the skin was stimulated electrically, with the usual result on the volume of the lung. At *B* the venom from four bees was injected intravenously. Occlusive bronchospasm resulted promptly, the oscillations of the lever being due chiefly to cardiac contractions. At *C* the insufflation pressure was increased markedly to prevent asphyxia, with little immediate effect on pulmonary excursions. At *D* the experiment was repeated on the same animal (ten minutes later).



Figs. 1-7

In order to elucidate the nature of the depressor action of the venom of bees a plethysmographic study of the kidney was made on an etherized dog weighing 6.8 kgm. After an intravenous injection of the venom from ten bees, the blood pressure fell precipitately to about 50 mm. of mercury. Simultaneously, the volume of the kidney showed a striking reduction. At the end of ten minutes the blood pressure began to rise slowly and in the course of an hour both the blood pressure and the volume of the kidney had regained nearly the original level. A second injection of the same size as the first was given, and a depressor response followed, which was accompanied by a decrease in the volume of the kidney, but the results following the second injection were less striking than those following the first (fig. 3). A sample of blood taken at the end of the experiment failed to clot after standing for fifteen minutes, but when examined after ninety minutes, it was found firmly clotted. Examination of the contents of the intestinal tract revealed quantities of blood. The serosa was a reddish-brown and the splanchnic organs were congested diffusely. The inference that splanchnic dilatation is not the cause of the immediate fall in blood pressure conforms with the observation that the injection of the venom from ten bees into a dog weighing 10 kgm. was followed by an immediate marked rise in the volume of the hind limb (fig. 4).

As might be expected, the intradermal injection of a dilute solution of the venom of the bee into the skin of a human being is followed by immediate reddening, whealing and a surrounding arteriolar flare.

In accordance with our investigation of the properties of the venom of rattlesnakes, we investigated the influence of the venom of the honeybee on the isolated heart of the rabbit when perfused according to the method of Locke and Rosenheim. The venom from eight bees was added to the perfusing fluid (200 cc.) and almost immediately the heart was noticeably affected; at the end of fifteen minutes its pulsations were too feeble to lift the recording lever (fig. 5).

Following these experiments the uterus of a virgin guinea pig was perfused by Dale's method. The preparation was perfused in 40 cc. of Ringer-Locke's solution. When the venom from one bee was mixed with the perfusing fluid maximal contraction occurred immediately. It required five minutes for the relaxation of the uterus after the perfusing fluid had been changed three times. A second dose of the same amount produced identical results (fig. 6).

This effect of the venom of the bee on the perfused uterus of the virgin guinea pig is quite in agreement with the effect of the venom on the bronchioles of the same species. A large guinea pig was injected with curare intravenously. Almost immediately the animal demonstrated the typical immobility. Under local anesthesia a cannula was rapidly placed in the trachea and artificial respiration was maintained by rhythmic insufflation

of oxygen at a pressure of 10 mm. of mercury. The excursions of the lungs were measured by recording the intrapleural pressure by means of a trocar which communicated with both pleural cavities. This method of measuring the volume of the lung was an adaptation of that described by Koessler and Lewis. When the venom from four bees was injected intravenously in such a preparation there was almost immediately a cessation of the movements of the piston recorder, indicating occlusive bronchospasm. When death seemed imminent the pressure of the insufflated oxygen was increased until respiratory excursions of the lung were manifested. It required an insufflation pressure of 80 mm. of mercury to bring this about. In ten minutes the preparation showed reasonably extensive pulmonary excursions with an insufflation pressure of 10 mm. of mercury. At this point, the venom from four bees was again injected intravenously. Occlusive bronchospasm again resulted (fig. 7).

The effect of the venom of the honeybee on the blood of the dog appears to be identical with that produced by the venom of the rattlesnake. If one adds 0.3 of the venom of one bee to 15 cc. of heparinized blood in a centrifuge tube, the diffusion of the venom through the blood is followed by a striking change in color, which immediately becomes a lighter red. After centrifuging at 2,500 revolutions a minute for twenty minutes, the ratio of corpuscles to plasma will be approximately 10 of corpuscles to 5 of plasma. A control specimen of blood centrifuged simultaneously will show a ratio of corpuscles to plasma that is approximately 6:9. Considerable hemolysis is always present in the tube containing venom of the bee and the plasma is also very turbid. The same results occur when blood is examined before and after the injection of the venom in a dog.

Comment. We have not considered fully the mechanism of the depressor action of the venom of the honeybee but have contented ourselves with the observation that an abdominal organ like the kidney diminishes in volume and a muscular organ like the hind limb increases in volume during the fall in blood pressure. As is the case with the venom of rattlesnakes, it seems that the immediate depressor action of venom of honeybees is due to extensive loss of blood into the skeletal muscle of the animal, with ultimately the enhancing factor of splanchnic dilatation. In the case of venom of bees, our belief that this is the mechanism of its depressor action is based on the remarkable similarity in the pharmacologic behavior of the two venoms rather than on an actual demonstration such as we reported for the venom of rattlesnakes. That the depressor action of the venom of honeybees is essentially peripheral is logical in view of the familiar local reaction following the bee's sting. It is the custom to designate such a local reaction as due to a "capillary poison," of which histamine is the best known example.

In general, the venom of the bee may be described as a violent endo-

thelial poison and a marked stimulant of smooth muscle; it is comparable in these respects to histamine. In view of the fact that the perfused mammalian heart is promptly incapacitated by this substance and erythrocytes are quickly laked, it would be preferable to designate the venom as a general protoplasmic poison, as we have done for the venom of rattlesnakes.

Occasionally following the intravenous injection of the venom of bees into a dog there is a preliminary rise in blood pressure (fig. 1). It is possible that some component other than the active principle of the venom is responsible for this initial rise. Apart from this observation, the venoms of the honeybee and of the rattlesnake apparently have similar physiologic properties. It is remarkable that the venom of the honeybee should be similar to that of the rattlesnake. Wasps, hornets, scorpions, mosquitoes, nettles, and other forms, whose sting is followed by a similar reaction, possibly will be shown to possess venom with similar properties.

SUMMARY

When the venom of honeybees is injected intravenously into dogs and rabbits it induces a rapid fall in blood pressure. Accompanying the fall in blood pressure the volume of the kidney diminishes and the volume of the hind limb increases.

The perfused uterus of the virgin guinea pig shows maximal contraction when the venom is added to the perfusing fluid.

When the venom is injected intravenously in guinea pigs occlusive bronchospasm results.

When the venom is injected intradermally into the skin of human beings a reaction is obtained that is practically identical with that produced by the venom of rattlesnakes and histamine.

The venom of the bee is markedly hemolytic, the process of hemolysis being preceded by a large increase in the volume of the erythrocytes.

The isolated perfused heart of the rabbit is rapidly incapacitated when the venom is added to the perfusing fluid.

These reactions closely approximate those that have been described for the venom of rattlesnakes.

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THE EFFECT OF CONTINUED STIMULATION AND OF SLEEP UPON CONDITIONED SALIVATION

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In the usual scheme of experimentation on conditioned reflexes the application of the conditioned stimulus is always discontinued when, or before, the unconditioned stimulation begins. In the morphine-induced conditioned salivation studied in our laboratory (Kleitman and Crisler, 1927) this was not the case. The dogs used had permanent fistulae of the duct of one of the submaxillary glands, and this permitted the study of the salivary response quantitatively. They were placed in stands, mainly to insure the proper collection of saliva, and they remained in these stands throughout the entire test. A definite constant time after the dog was placed in the stand he was given a subcutaneous injection of morphine, which generally resulted in nausea, salivation, vomiting and finally a state of depression. Repetition of this procedure once a day for several days resulted in a conditioned salivation becoming gradually established, the animals salivating on being placed in the stand, before the injection of morphine. Here instead of a definite simple conditioned stimulus we had a complex of tactile and visual stimuli resulting from *being* in the stand. But, as indicated above, the dogs remained in the stand after the morphine was injected, and so we have in this a case of continued action of the conditioned stimulus after the application of the unconditioned one. This is a novel situation in conditioned reflex work, and the reason the effect of the continued action of the conditioned stimulus-complex could not be observed was that the depressive action of morphine rendered the dogs practically insensible to events in their surroundings.

The salivary secretion after the injection of morphine followed a definite curve depending mainly on the secretory level maintained before the injection, but it always came down to zero in a short time. The average duration of the salivary flow after the injection varied from 15 to 20 minutes, in different dogs. Although it was generally referred to as unconditioned salivation, the post-injection flow of saliva was undoubtedly influenced by the rate of conditioned salivation that preceded the injection of morphine.

The only opportunity we had of observing the effect of continued conditioned stimulation was in the case of a dog which seemed to be less affected

by morphine than the other animals studied. All the observations to be reported were made on this one dog. As a rule, one hesitates in making conclusions on the basis of results from only one animal, but in this dog the same striking results were obtained with such regularity on so many occasions over a period of several months, that they appear to be of significance in demonstrating the effect of continued action of the conditioned stimulus.

The dog (female) was one of a number used in the study of morphine-conditioned salivation, and at first she behaved as the other dogs did. The injection of 30 mgm. morphine sulphate caused the usual symptoms, including salivation lasting for from 10 to 25 minutes. As the conditioned secretion appeared, a definite prolongation of the post-morphine secretion appeared with it. When the conditioned secretion reached its full development, the flow of saliva after the injection continued as long as the dog remained in the stand. On one occasion the dog was left in the stand for 4 hours 35 minutes, and she secreted from the fistula alone 169.6 cc. during that time; on another occasion, 194.1 cc. of saliva were secreted in 5 hours 40 minutes. On both of these occasions the saliva was still flowing well at the time the dog was taken out of the stand. Some of the saliva secreted by the other glands was swallowed, and some dropped from the mouth and was collected in a pan placed on the stand, directly under the dog's mouth. Without allowing for evaporation of water from the dog's lips and from the pan, the actual loss of water from the animal's body, the saliva collected from the fistula and the drippings in the pan, amounted to as much as 350 cc., or 3 per cent of the weight of the dog. That dehydration was sufficient to send up the red blood cells of the blood from 42–43 per cent to 47–48 per cent.

The curve of the post-morphine salivary secretion gives an indication of the nature of the process. It will be seen from figure 1A that the rate of secretion for successive five-minute periods is not uniform. In the beginning there is a progressive decrease, but after 25 to 30 minutes the rate of secretion gradually increases and continues with minor ups and downs for the rest of the experiment. On those occasions when the dog was left in the stand for several hours, it was found that only after about three hours of steady flow did there appear a tendency to a lower rate of secretion. The curve in figure 1A is interpreted as indicating that after a preliminary stimulation morphine produced a depression, but the depression soon wore off and the conditioned stimulus could begin to exert its action anew. To test this interpretation the dog was given alternately 30 mgm. and 60 mgm. of morphine. The curve of the average rate of secretion for successive five minute intervals after the double dose of morphine, given in figure 1B, shows a much greater fall and a slower and less marked recovery of salivary flow than the secretion curve after 30 mgm. of morphine. That there was a considerable recovery from the depressive effect of the morphine injection

was also shown by the fact that the dog invariably ate her meal after the test, which other dogs did but very seldom.

Another peculiarity exhibited by this dog was a tendency to fall asleep while in the stand, both before the injection of morphine and after the effect of the injection wore off. This tendency developed soon after the reflex was established, and when unchecked, it interfered quite seriously with the salivary flow. As drowsiness appeared, the secretion generally

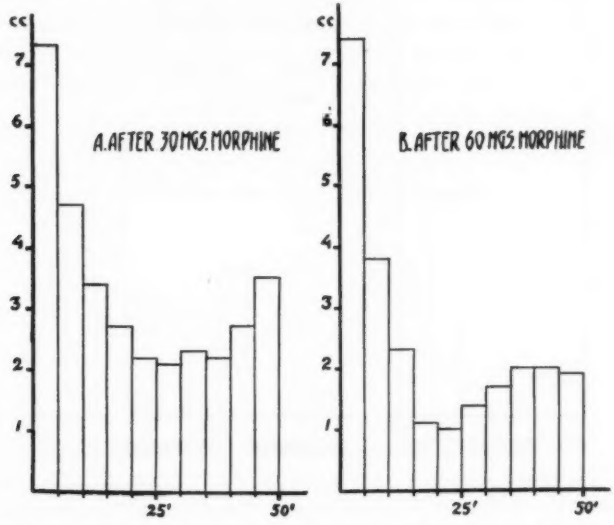

Fig. 1. The degree of depression and the extent of recovery of the conditioned salivation, following the injection of different doses of morphine. *A*. The average rate of salivary flow for successive five minute periods after the subcutaneous injection of 30 mgm. of morphine into a dog with a well established conditioned response to morphine. Based upon data from ten experiments. *B*. The average rate of salivary flow for successive five minute periods after the injection of 60 mgm. of morphine into the same dog under the same conditions. Based upon data from four experiments interspersed with those which furnished the data for *A*.

diminished, and with the onset of sleep it would stop altogether. Waking the dog resulted in a flare-up of the salivary flow, and the only way in which a steady uniform flow could be obtained was by keeping the dog awake. Walking around the room or talking to the dog was usually sufficient to prevent her from falling asleep. A typical example of the secretory activity of the dog is given in figure 2. The presence of another person in the room did not prevent the animal from sleeping, provided that the person was familiar to her. An attempt to demonstrate this phenomenon to individuals whom the dog did not know almost always

resulted in failure. Not only did the animal remain awake, but the flow of saliva was more copious than usual.

The behavior of this dog is not in agreement with the postulates of Pavlov's theory of sleep (1927). According to this theory, local cortical

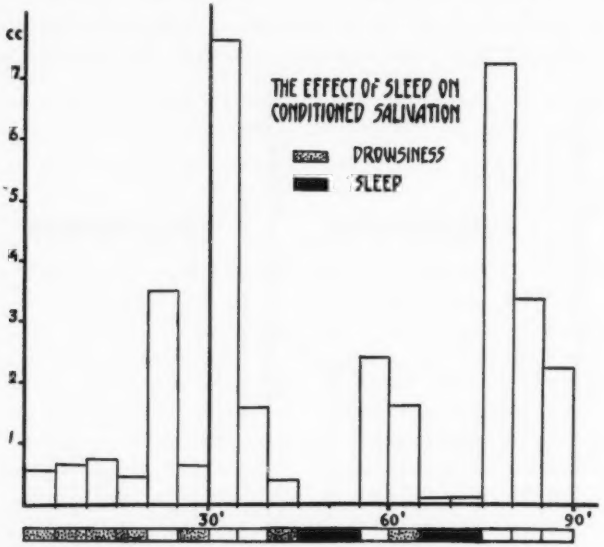

Fig. 2. A typical experiment showing the effect of drowsiness and sleep upon the rate of salivary secretion of a dog, for successive five minute periods, before and after the injection of morphine. During the first twenty minutes the dog was drowsy (eyes semi-closed) and the secretion rate was 0.4 to 0.7 cc. in five minutes. During the next five minutes the dog was kept awake, and the quantity of saliva secreted rose to 3.5 cc. Permitted to become drowsy again, the dog secreted only 0.6 cc. in the last five minutes before the injection. After the injection of morphine the dog secreted 7.6 cc. and 1.6 cc. for the first two five minute periods. She then became very drowsy, and the 0.4 cc. shown for the third period was really collected in the first two minutes of that period. During the fourth and fifth periods the dog was definitely asleep and snoring, no saliva coming out of the fistula for 13 minutes. The dog was then aroused and kept awake for the sixth period when she secreted 2.4 cc. Somewhat drowsy again during the seventh period, the secretion fell to 1.6 cc., and asleep during the eighth and ninth periods, when only 0.1 cc. was collected for each of these periods. Aroused again and kept awake, the secretion of saliva for the next three periods was 7.2 cc., 3.3 cc., and 2.2 cc. respectively.

inhibition is local sleep, and general sleep is generalized cortical inhibition. Sleep observed during the work on conditioned reflexes, Pavlov holds, follows an inhibition of a conditioned reflex, when the inhibition spreads over the entire cerebral cortex. In this dog drowsiness and sleep came

first and the cessation of the salivary flow followed, most probably because the action of the conditioned stimulus could not be exerted on the sleeping animal. That there was no inhibition of the conditioned reflex as such preceding the onset of sleep was indicated by the immediate resumption of the salivary flow when the animal was awakened.

Bearing in mind that these observations were limited to one animal and to one conditioned phenomenon, it appears that—

1. Application of the unconditioned stimulus does not prevent further response as a result of the continued action of the conditioned stimulus.

2. With a conditioned stimulus acting continuously, sleep cuts short the conditioned activity and awakening leads to its immediate resumption.

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THE REGULATION OF RENAL ACTIVITY

XII. The Relation of the Rate of Creatinine Excretion in the Urine to the Plasma Concentration[1]

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In the experiments described here, the relation of the rate of creatinine excretion in the urine to the plasma creatinine concentration has been determined. Rehberg (1926) has made such a comparison after creatinine administration to himself, but failed to maintain any degree of constancy in his conditions which may account for the fact that he found only a general tendency for the urine rate to increase with the plasma concentration. The present observations were made on rabbits under conditions which have been found (Addis, Foster and Barnett, 1918) to lead to full renal activity and not only make it possible to disregard the degree of renal activity, because it is constant, but which have been shown (Addis and Drury, 1923) to give a greater constancy of the factors other than the blood urea concentration which determine the rate of urea excretion. A priori these conditions would seem to be the most suitable for observing the relation of the rate of creatinine excretion to the creatinine concentration in the plasma.

Methods. Healthy male rabbits were selected for these experiments. No food was given for 15 hours before the experiment was commenced. Three hours before the first catheterization 30 cc. per kilo body weight of a 5 per cent solution of urea were given by stomach tube, and every hour thereafter until the experiment was ended 30 cc. of water per kilo were administered in the same manner. One to 5 grams of creatinine per kilo were either given by mouth with the water an hour after the urea solution was given or injected intravenously in 10 per cent solution an hour and a half later.

The urine was obtained by catheter at intervals of one-half hour. In all of the observations which are reported here the urine volumes were 5 cc. per kilo per hour or better. After each catheterization the bladder was thoroughly washed with distilled water. Blood specimens were ob-

[1] This investigation was made possible by the Edward N. Gibbs Prize Fund of the New York Academy of Medicine.

tained at the middle of each urine collection by heart puncture and centrifuged for serum or plasma. In case the latter was desired, a minimal amount of heparin, which does not crenate the red cells, was used. Creatinine was determined in the plasma and urine by Folin's methods and urea was determined in the urine by a urease and titrametric method and in the plasma by the usual aeration procedure (Addis, 1924).

Relation of the rate of creatinine excretion to the plasma concentration. Ten experiments, or series of observations, were carried out in the manner we have described. The results of three typical experiments have been plotted in figure 1. There were good urine volumes throughout in each case. The observations of Behre and Benedict (1922) concerning the question of whether or not creatinine is normally present in the plasma can have little influence upon our results for the plasma concentrations were raised so much by the administration of creatinine that the strength of the normal creatinine reaction of the plasma becomes insignificant.

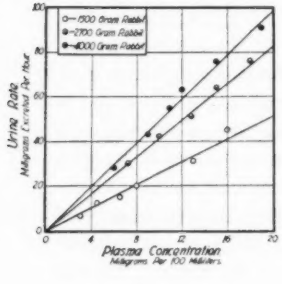

Fig. 1. Creatinine

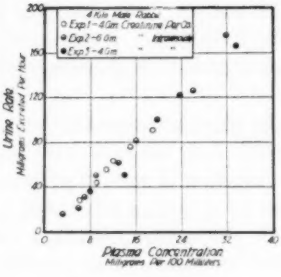

Fig. 2. Creatinine

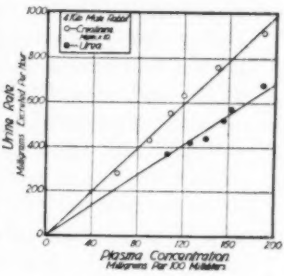

Fig. 3. Creatinine and urea

Figure 1 shows that under certain conditions the rate of creatinine excretion in the urine is directly proportional to the plasma creatinine concentration. There are differences in the rate at a given plasma concentration in the three animals but, as in the case of urea (Taylor, Drury and Addis, 1923), these are undoubtedly due to differences in the amount of renal tissue which they possess. When the procedure is repeated upon the same rabbit the same relationship is obtained. This is shown in figure 2. Gonteyne and Ingelbrecht (1928) compared the urine rate with the plasma concentration after the injection of 1 gram of creatinine in a dog. Their data suggest a straight line relationship but cannot be correlated by means of a direct linear formula and therefore indicate a threshold. Our results do not show this.

Comparison of the rate of creatinine and urea excretion. Under the conditions for which we have found the rate of creatinine excretion to be

directly proportional to the plasma concentration, the rate of urea excretion likewise bears a direct relation to the plasma urea concentration (Drury, 1923). With this in mind, the excretion of these two substances has been observed simultaneously. They are compared in figure 3. This is a typical experiment, one of four similar series of observations. Although the rates of excretion of both urea and creatinine are directly proportional to their plasma concentrations, the rate for a given urea figure is consistently lower than for a similar creatinine concentration, that is, the excretory ratio: $\frac{\text{Urine rate}}{\text{Plasma concentration}}$ for creatinine is higher than that for urea.

DISCUSSION. By direct experiment it has been shown that phenol red (Oliver and Shevky, 1929) and urea (MacKay and Oliver, 1930) are excreted in the frog's kidney by glomerular filtration. Their behavior in the mammalian kidney in comparison with a substance which in the frog is secreted by the tubules is such that there is every reason to suppose (MacKay and Oliver, 1930) that they are also excreted by the mammalian kidney through glomerular filtration. A characteristic then which might place a substance as being excreted by glomerular filtration would be that under conditions of full renal activity the rate of excretion in the urine is directly proportional to the plasma concentration, since it has been shown (Mackay and Oliver, 1930) that when a substance is secreted by the tubules there is no direct relation of rate to plasma concentration. Creatinine has here been shown to be such a substance. If both creatinine and urea then are excreted by glomerular filtration and there is no tubular reabsorption of either, their excretory ratios should be the same. This is not the case, creatinine having a notably higher excretion rate at a given plasma concentration. We must assume therefore that additional creatinine is excreted by the renal tubules or that there is a substantial reabsorption of urea in the tubules. The latter view would seem most likely to be the correct one. Mayrs (1922) came to a similar conclusion through a study of concentration ratios and evidence has been obtained by indirect methods (MacKay and MacKay, 1930) which suggests very strongly a diminution in tubular reabsorption of urea following blockage and damage to the cells by the deposition of large amounts of dye. If urea is excreted by glomerular filtration and there is some reabsorption by the tubules the reabsorption rate under the conditions discussed here must be directly proportional to the blood urea concentration, and, secondarily, to the observed urine urea rate.

SUMMARY

Under certain special conditions which it is reasonable to suppose lead to full renal activity creatinine excretion has been shown to be similar to

urea excretion in that the urine rate is directly proportional to the plasma concentration. The excretory ratio: $\frac{\text{Urine rate}}{\text{Plasma concentration}}$ is consistently higher for creatinine than for urea. The significance of this is discussed.

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THE REGULATION OF RESPIRATION

The Effects of Hemorrhage and Reinjection, and of the Intravenous Injection of Sodium Carbonate and Sodium Cyanide upon the Response of the Neuro-muscular Mechanism

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The experiments described in this paper were designed to study the effects of hemorrhage, reinjection, and the injection of Na_2CO_3 and NaCN upon the contractions of the sartorius muscle of the dog in response to peripheral motor nerve and direct muscular stimulation. These experiments were performed with the animals intact, the blood supply and innervation of the muscles studied being disturbed as little as possible. They were undertaken primarily to supplement the observations of Glazer (1929) and of Winkler (1929), who found certain changes following these same procedures in the muscular response to reflex stimulation, but were unable in most instances to ascertain the point in the reflex arc at which these changes were localized. It was hoped that by a comparison of the results obtained in these experiments, using stimulation of the peripheral part of the arc only, with those previously obtained using reflex stimulation, it would be possible to localize to some extent the portion of the arc responsible for the changes observed. In order to carry this analysis of the reflex arc one step further, in every experiment a simultaneous record was made of the response of one sartorius muscle to motor nerve stimulation and of the other to direct muscular stimulation. Thus by a comparison of the changes occurring in each response it should be possible to decide whether the nerve and neuromuscular junction, or the muscle fibers themselves, or both, were responsible for the effects observed.

In addition to these more immediate objects of this group of experiments, it was hoped that some light might be thrown upon the peripheral chemical regulation of the respiratory muscles. With this end in view a simultaneous record of the pulmonary ventilation was made.

Method. The method used was essentially that of Glazer (1929), as modified by Knoepp and Gettel (1929). The dog was anesthetized with morphine and urethane, and firmly tied to the dog board. The sartorius muscle on each side was severed at its insertion and attached to a muscle lever, which was so arranged that the muscle pulled against a spring under

tension. On one side the motor nerve to the sartorius muscle was isolated, crushed, and ligated and a pair of shielded electrodes were applied below the ligature; on the other side a special electrode, consisting of two strips of platinum mounted parallel to each other on a flat bakelite base, was slipped underneath the lower third of the muscle. This part of the muscle is reported to contain few, if any, motor nerve endings, and so it was assumed that by stimulating in this fashion only the muscle fibers themselves would be affected. Stimulation was obtained from the secondary coil of an inductorium, in the primary circuit of which was placed a specially constructed circuit breaking device, which operated regularly about once a second, and which short circuited all make shocks, thus allowing only break shocks to pass through and cause stimulation. The stimulation was very constant, the height of muscular contraction normally remaining unchanged for considerable periods of time. Both submaximal and supramaximal stimuli were used.

Respiration was accurately recorded by the use of a closed circuit rebreathing tank connected to the trachea, a soda lime cartridge being placed in the circuit to absorb CO_2. Blood pressure was recorded from one carotid, the other being used for hemorrhages. All injections were made into one of the jugular veins. Time was recorded in seconds.

RESULTS. These experiments were performed on a series of nineteen dogs, ranging from ten to twenty kilos in weight. Two groups of experiments were performed, in one of which (group A) submaximal stimuli, in the other (group B) supramaximal stimuli, were employed. An attempt which was, however, only partially successful, was made to perform both groups of experiments on each animal.

Group A: Submaximal stimuli. (Fig. 1A to, 11A.) Hemorrhage was in general followed by a decrease in amplitude, both in the response to the motor nerve and to the direct muscular stimulation; reinjection of defibrinated blood or of glucose saline was, on the other hand, usually followed by an increase (fig. 1A, 2A). Effects definitely the reverse of these were never noted, but animals varied considerably in the degree of response to a hemorrhage or reinjection of a given size.

These results stand out in definite contrast to those previously obtained with the reflex response after hemorrhage and reinjection (Winkler, 1929). As described in this paper the effects usually observed when the muscle was stimulated reflexly were an increase after hemorrhage and a decrease after reinjection, i.e., the muscle tended to vary in a direction the reverse of that in the experiments using peripheral stimulation. Also, in contrast to the very constant results obtained with peripheral stimulation, it may be noted that these reflex response changes after hemorrhage and reinjection were by no means invariable, changes in the reverse direction being at times observed.

Sodium carbonate was injected intravenously in amounts varying from 25 to 50 cubic centimeters of a 10 per cent solution. Ordinarily injections of this size produced an increase in both the motor nerve and in the direct muscular responses (fig. 4A, 5A). The duration of this increase varied considerably, sometimes passing off rather quickly (fig. 4A); at other times continuing for a considerable period (fig. 5A). In a certain number of cases the increased response occurred in the response to the motor nerve but not to the direct muscular stimulation (fig. 3A, 6A, 7A). The particular form which the increase took varied considerably. Thus in figure 6A the increase is apparently accompanied by certain tonus changes (although a slight twisting of the whole animal may be responsible in part for this appearance). In figure 8A we see a case in which there is a temporary cessation of the response to motor nerve stimulation subsequent to the injection, followed by the usual increase; and in figure 3A the amount of carbonate is sufficient to send the animal into a tetanic spasm for the moment, thus causing a decrease in length of the muscle together with a great decrease in the amplitude of contraction during the period of the spasm.

In respect to the fact that an increased muscular contraction in response to this peripheral stimulation could be obtained after injection of Na_2CO_3, the effect resembles that obtained by Glazer (1929) in the case of the response to reflex stimulation. But there are important differences. Glazer with great constancy was able to produce a very definite increase in the height of the reflex response by the injection of from five to twenty cubic centimeters of 10 per cent Na_2CO_3. In the experiments with the peripheral nerve and muscle stimulation, on the other hand, very little if any effect could be obtained with injections of this size. Very rapid injections, varying in size from twenty five to fifty cubic centimeters, were required to produce an increase. Even with excessively large amounts the increase sometimes failed to occur, or was very slight and transient. Thus in general it may be said that this increase in response to peripheral stimulation was obtainable only with much larger injections and with much less constancy than in the case of reflex stimulation, as studied by Glazer.

Sodium cyanide when injected intravenously was in general found to produce an increase both in the response to motor nerve and direct muscular stimulation. This is typified in figure 9A, in which there is a rise in both responses after each injection, which maintains itself for a few moments, and then gradually recedes to normal and even below. In figure 11A, however, we see a case in which there is a sustained increase in the response to motor nerve stimulation (together with slight tonus changes), but no accompanying changes in the direct muscular response. Furthermore, decreases in one or both responses were very occasionally noted as well. Thus in figure 10A a slight falling off of both responses is the only result of the first injection; after the second injection there is a very sudden

increase, which very rapidly tapers off below the normal level, only to make a partial recovery at the end of the record. This occurrence of an immediate primary increase followed later by a period of decreased activity was not infrequently observed. It may be stated that in general the increase in muscular response to direct stimulation was obtained with greater ease and constancy than in the case of Na_2CO_3: to bring about this increase only 3 to 8 cc. of a M/50 solution were required, an amount comparable to that used by Glazer to elicit a similar increase in his experiments on reflex stimulation.

Group B: Supramaximal stimuli. The effects of hemorrhage and reinjection were similar to those obtained in the case of submaximal stimuli. Hemorrhage always produced a decrease, and reinjection of defibrinated blood an increase (fig. 1B).

The effects of Na_2CO_3 on the response to motor nerve and muscular stimulation were somewhat uncertain. In the majority of cases no definite change occurred. Sometimes, however, a slight increase followed (fig. 4B, especially after the second injection), but this effect was never marked nor was it obtainable with any degree of constancy. Even amounts of carbonate sufficient to cause a tetanic spasm of all the muscles of the body were usually insufficient to cause any definite change (fig. 3B). Figure 2B shows a case in which there was a slight decrease in the response to motor nerve stimulation after the first injection, followed by a prompt return to normal, the response to direct muscular stimulation remaining unchanged; but the second injection produced no apparent effect on either response. In a few other cases a similar transient decrease was noted, but in these instances the decreased muscular contraction might readily be explained by the decreased nutrient flow through the muscle, attendant upon the simultaneous pronounced and lasting drop in blood pressure which in these particular cases usually accompanied the diminished muscular activity. So in general the experiments performed do not warrant any more conclusive statements as to the effect of Na_2CO_3 than that moderate quantities were usually without effect, and that the effects of larger quantities were most uncertain.

The effects of NaCN in this group of experiments were rather different from those occurring with submaximal stimuli, a decrease in the response to motor nerve and to direct muscular stimulation being the usual result of moderate quantities of cyanide (fig. 5B, 7B, 9B). Subsequently there was a partial or complete return to normal (fig. 5B, 7B). It seemed as if this decrease occurred more readily in the case of the direct muscular stimulation than of the motor nerve; at least a decrease in the former not infrequently occurred without change in the latter (fig. 6B, and 7B first injection). With regard to the effects of smaller quantities of cyanide the results obtained, as in the experiments with Na_2CO_3, were ambiguous.

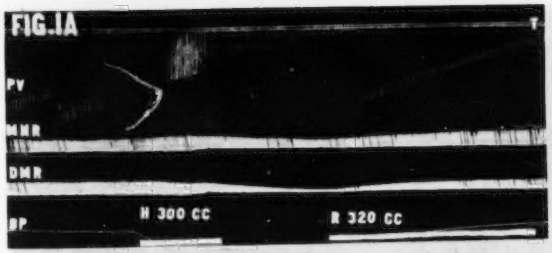
FIG.1A
PV
MMR
DMR
BP
H 300 CC
R 320 CC

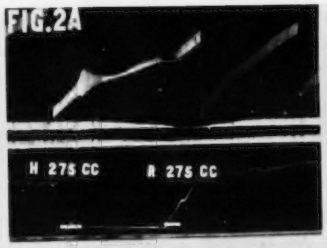
FIG.2A
H 275 CC
R 275 CC

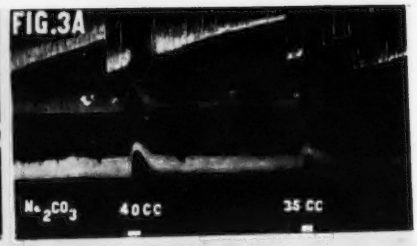
FIG.3A
Na_2CO_3
40CC
35 CC

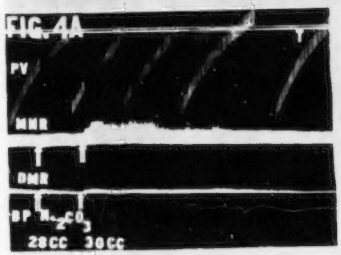
FIG.4A
PV
MMR
DMR
BP Na_2CO_3
25CC 30CC

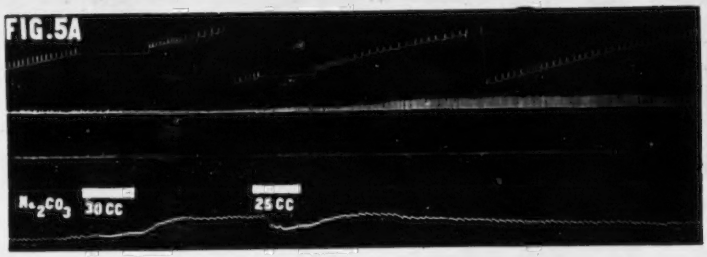
FIG.5A
Na_2CO_3 30CC
25CC

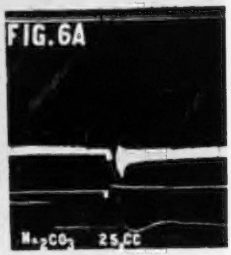
FIG.6A
Na_2CO_3 25CC

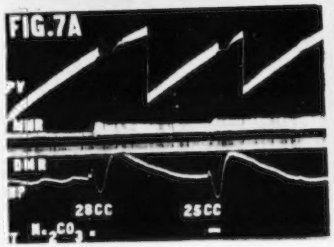
FIG.7A
PV
MMR
DMR
BP
25CC
25CC
Na_2CO_3

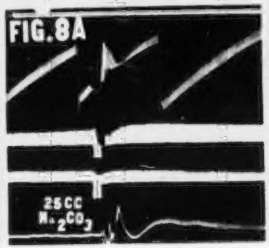
FIG.8A
25CC
Na_2CO_3

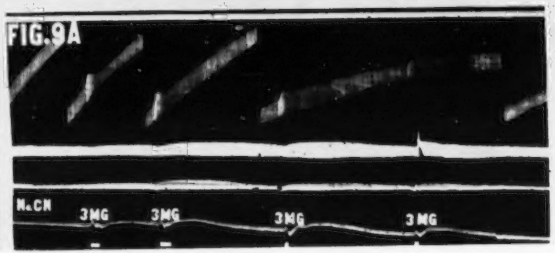
FIG.9A
NaCN
3MG
3MG
3MG
3MG

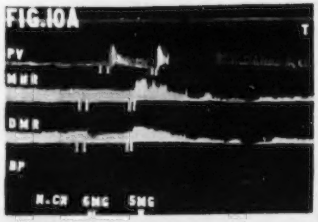
FIG.10A
PV
MMR
DMR
BP
N.CN 6MG 5MG

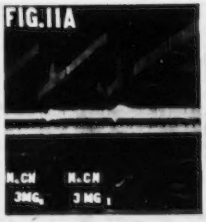
FIG.11A
N.CN N.CN
3MG, 3MG,

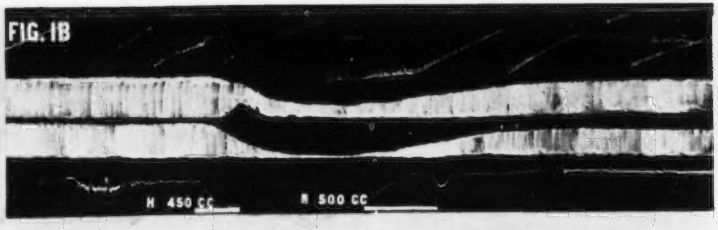
FIG.1B
N 450 CC
R 500 CC

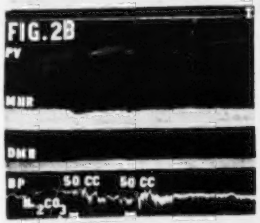
FIG.2B
PV
MMR
DMR
BP 50 CC 50 CC

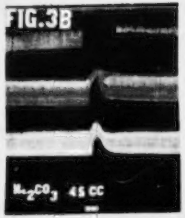
FIG.3B
45 CC

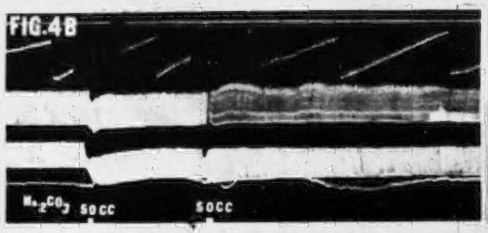
FIG.4B
50 CC
50CC

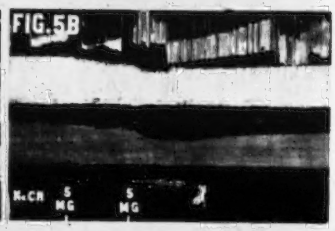
FIG.5B
N.CN 5 5
MG MG


FIG.6B
PV
MMR
DMR
BP
N.CN 5 5
MG MG

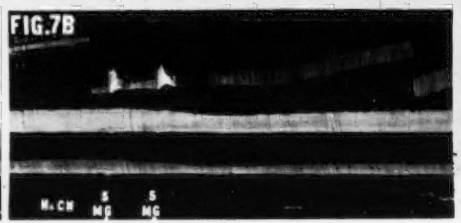
FIG.7B
N.CN 5 5
MG MG


FIG.8B
N.CN 5 5
MG MG

Most commonly no change at all resulted. Sometimes an apparent slight increase occurred in the response to motor nerve stimulation (fig. 6B), and less commonly an increase in the response to direct muscular stimulation (fig. 8B, first injection). But never were these increases very marked, not could they be obtained with any degree of constancy.

In all the experiments of both series summarized above the records were carefully examined in order to discover if possible the existence of any correlation between the changes in muscular activity and changes in the activity of the respiratory muscles, as reflected in changes in the pulmonary ventilation.

In the case of hemorrhage and reinjection the mass of evidence goes to show that no such correlation exists. Thus in figure 2A the hemorrhage is followed by a brief period of marked hyperpnea, then by a period of reduced ventilation, and finally some increase again, whereas the muscular responses decrease uniformly and rather gradually from the start. Reinjection is followed by a brief period of apnea, and then a rather sudden return of the respiration to normal rate and depth; the muscular responses on the other hand gradually increase, these changes bearing no immediate relation to those occurring in the respiration. Figure 1A represents the only case in which one might suspect the existence of some parallelism. Here the hemorrhage is followed by a brief period of hyperpnea, and running parallel with this is a very slight increase in the motor nerve response; this lasts but a moment, and then is followed by the usual drop. The response to direct muscular stimulation shows no such preliminary increase, however; and subsequent injection had its most usual effect, viz., an increase in both muscular responses together with a decrease in pulmonary ventilation. The rare occurrence of such an apparent correlation as occurred during this hemorrhage coupled with the very slight changes involved, makes it unwise to lay too much stress upon such an isolated finding.

With carbonate injections likewise the general rule was absence of correlation, since the usual effect of carbonate was a diminution in respiratory movements accompanying an unchanged or increased muscular response. Thus in figure 4A each injection results in a brief period of apnea, followed by an immediate return of the respiration to normal; but the response to direct muscular stimulation is increased after each injection, and the response to motor nerve stimulation, though practically unchanged after the first, is markedly increased after the second injection. In figures 6A and 7A likewise the periods of diminished respiration after each injection are accompanied by increased muscular activity, while in figure 2B the long period of apnea after the second injection is accompanied by no change whatsoever in the muscular response. Indeed the only suggestion of a possible correlation appears in figure 8A, in which the carbonate injection is followed first by a very short period of apnea, then by a very marked

hyperpnea, next a period of diminished activity for a few seconds, and finally a complete return to normal. The muscular response in a somewhat similar fashion first markedly decreases and then very rapidly increases above normal, this increase then persisting for some time. However, the muscular changes though in the same direction as the respiratory changes are out of proportion to them, both in degree and in duration, so that even here we cannot be sure of any true parallelism.

In the case of cyanide injections there is more evidence, in the experiments with submaximal stimuli, in favor of a degree of correlation, since in this group the typical results of cyanide injection were simultaneous increases both in respiratory and in muscular activity. Thus in both figures 9A and 11A the correlation is apparently distinct; following each injection of cyanide there is a short period of marked hyperpnea, followed by a more prolonged period in which the pulmonary ventilation is distinctly increased above the previous level; and running parallel with these changes the muscular activity is first markedly increased and then falls off slightly, but continues for some time at a level greater than that observed before the injection. However, this correlation does not always occur, as may be seen in figure 10A. Here the first injection produced a marked hyperpnea, yet the muscular responses show no parallel increase, the response to direct muscular stimulation indeed actually decreasing. Though the hyperpnea following the second injection is accompanied by an increased muscular activity, the subsequent rapid falling off of the muscular responses is not paralleled by any similar change in the pulmonary ventilation. In the group of experiments using supramaximal stimuli lack of correlation was the rule, since the muscular response was here typically decreased or at best remained unchanged, while the respiration as usual was characteristically increased. Thus figure 7B shows a marked hyperpnea after each injection and a subsequently permanent increase in respiration, while the muscular responses gradually decline and then slowly return to normal.

In reviewing the records any possible correlation with changes in blood pressure was incidentally noted. It was found that in the case of hemorrhage and reinjection a very definite correlation existed (fig. 1A, 2A, 1B), the fall in the muscular responses after hemorrhage running parallel with the fall in blood pressure, and the rise after reinjection with the rise in blood pressure. In the case of cyanide the blood pressure sometimes rose (fig. 8B) and sometimes fell (fig. 7B), and there is no apparent correlation between the muscular response and these changes. Carbonate typically produced first a brief period of irregularity, a sudden rise, a fall, a rise again, and finally a gradual drop of the blood pressure to normal (fig. 7A). Again, it was not possible to correlate these with muscular changes.

Discussion. It will be noted that the two series of experiments, using

submaximal and supramaximal stimuli respectively, serve to measure quite distinct phenomena. In the case of submaximal stimuli we are studying primarily the variations in the threshold of stimulation, i.e., the excitability of the preparation. In the case of supramaximal stimuli, on the other hand, we are concerned only with the contractility. It must be noted, however, that in the case of submaximal stimuli variations in the contractility of the muscle enter into the end result actually observed as well. Thus, a decrease in the contractility and a simultaneous increase in excitability would tend to oppose one another, and the final resultant amplitude of contraction would depend upon the relative changes in these two factors. This complication presumably does not occur in the case of supramaximal stimuli.

Before proceeding to a discussion of results we may point out one conclusion which applies with equal force to all the groups of experiments. It will have been noted that in every case studied the response to motor nerve stimulation and to direct muscular stimulation in general varied in the same direction. No significant exceptions to this statement were found. Hence it follows logically that such important changes as were observed must occur within the muscle fibers themselves as well as in the motor nerve and neuromuscular junction. Indeed it may possibly be that changes in the muscle fibers are wholly responsible for the changes observed.

In comparing the results obtained in these experiments using peripheral stimulation with those previously obtained using reflex stimulation by Glazer (1929) and Winkler (1929), certain conclusions seem apparent. Thus, the results obtained in the experiments with hemorrhage and reinjection serve to confirm and extend the findings of Winkler (1929), discussed previously. On the basis of these previous findings the conclusion had been reached that the changes in the reflex response following hemorrhage, being in the opposite direction to the peripheral changes, were, therefore, most probably of central origin. The experiments in this study confirm this previous suggestion that the increased reflex response after hemorrhage occurred in spite of peripheral changes, and thus represented a true increase in the excitability of the cord. However, no experiments have been undertaken to study variations in the response of the afferent fibres of the reflex arc, so that central localization although very probable cannot be said to be certain.

Carbonate injections in general produced an increase in excitability of the neuromuscular mechanism together with an absence of change in contractility. But, as was discussed before, the amount of carbonate necessary to produce an increase was so much larger than that needed by Glazer to obtain a similar increase in the reflex response, that it is difficult to believe that these changes in the reflex response were wholly due to this increased peripheral excitability. Rather it would seem likely that there

was a central effect as well, tending to act in the same direction and to reënforce the weaker peripheral effect demonstrated here.

Sodium cyanide produced an increase in excitability and at the same time a decrease in contractility, the increase in excitability usually predominating. Glazer (1929) found an increase in the reflex response following cyanide injections similar to that usually observed in our experiments using peripheral stimulation. The amount of cyanide required was of the same order of magnitude as in his experiments, so that it is just possible that the results obtained by him were wholly due to these peripheral changes. However, the contrast between the ease with which the reflex response could usually be augmented by small quantities of cyanide, and the difficulty so often encountered in these experiments using peripheral stimulation in causing any change at all, was so striking that little doubt remains but that there exists a central effect as well which tends to strengthen and augment the peripheral effect in the muscular response to reflex stimulation.

SUMMARY

A series of experiments is described in which are studied the effects of hemorrhage, reinjection, and the injection of sodium carbonate and sodium cyanide upon the amplitude of contraction of the sartorius muscle of the dog. Both submaximal and supramaximal stimulation of the motor nerve and of the muscle were employed.

The simultaneous responses to motor nerve and to direct muscular stimulation in general showed similar variations.

Hemorrhage, if large enough, was found to produce a decrease in amplitude, reinjection an increase, the effect being the same whether submaximal or supramaximal stimuli were used.

Sodium carbonate when injected in large quantities was found to produce an increase in the response to submaximal stimuli. Smaller quantities were without effect. The effect of carbonate on the response to supramaximal stimuli was inconstant. In general no significant changes occurred.

Moderate injection of cyanide produced an increase in the response to submaximal stimuli, sometimes followed by a later decrease. Cyanide in larger quantities produced a decrease in the response to supramaximal stimuli. The effect of lesser quantities was uncertain.

Some correlation between changes in pulmonary ventilation and those in muscular activity after cyanide was observed; such a correlation was not found with the other procedures.

The relation of these experiments to the previous work of Glazer and Winkler was discussed. The conclusion was reached that the previously studied increase of the reflex response after hemorrhage and decrease after

reinjection were probably wholly central in origin; the reflex increase after carbonate probably both central and peripheral, but mainly central; and the reflex increase after cyanide certainly peripheral in part with possibly a central component as well.

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THE MECHANICAL THRESHOLD OF THE CORNEA-REFLEX OF THE USUAL LABORATORY ANIMALS

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The cornea-reflex of animals as well of man is often used as an indicator for the estimation of the degree of narcosis. This reflex could be made useful in a still more far-reaching extent for pharmacological and pathological-physiological purposes, by applying stimuli of definite quality and strength. In the present paper I wish to give a report of some experiments carried out in this way on the usual laboratory animals, experiments I have performed in the laboratory of Doctor Carlson, where I had the privilege of spending some time as fellow of the Rockefeller Foundation.

METHOD. For the stimulation of the cornea I used v. Frey's test hairs (Reizhaare). The hairs were gauged in pressure-values in terms of g/mm^2, that is to say, according to the ratio between the maximal resistance and the area of the cut hair. Hairs possessing the following pressure-values, were used: 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0 a.s.o. g/mm^2.

The hair is very carefully placed on the middle of the cornea and forced into a slightly bowed position and maintained in this position for about three seconds. This procedure is repeated ten times with each of a series of hairs; the pressure of that hair, which produces a lid response following 50 per cent of the stimulations, is designated as the threshold value of the cornea reflex. It is advisable to regard only decided responses for the determination of the threshold. The experiments were made on 10 guinea pigs, 10 rabbits, 10 rats, 9 pigeons, 6 dogs, 3 cats and 3 monkeys.

RESULTS. *Guinea pigs.* Table 1 gives the threshold values of the cornea reflex of *10 guinea pigs.* Animals 1 and 2 were young pigs, and 3 to 10 were adult animals. For the young animals the threshold values ranged between 14 and 20 g/mm^2 and for the adult animals these values ranged between 8 and 18 g/mm^2. The average threshold value for the right eye of the older animals was 12.3 and for the left eye 11.4 g/mm^2, so that the *average value* for this species of animals can be designated *as about 12 g/mm^2.*

Rabbits. Adult rabbits showed variations for the right eye between 8 and 11 g/mm^2 (see table 5, nos. 4–10). Rabbits of about three months of age

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showed somewhat lower threshold values (see table 5, nos. 1–3). Excluding the younger animals, the *average threshold* value amounts *to 9 g/mm²*.

Dogs. Six normal *dogs* varying in age from 2 to 7 years, had for the right eye an average threshold value of 4.5 and for the left eye 4.3 g/mm². These values were calculated from individual values ranging between 3 and 5 g/mm² (table 2). Dogs are not especially favorable animals for experiments of this kind because of the frequent voluntary movements of

TABLE 1

GUINEA PIGS NUMBER	THRESHOLD IN G/MM²	
	Right eye	Left eye
1	18	20
2	20	14
3	18	14
4	18	15
5	15	15
6	13	10
7	13	11
8	13	8
9	9	8
10	8	10
3 to 10	12.3	11.4

TABLE 2

DOGS	AGE IN YEARS	THRESHOLD IN G/MM²	
		Right eye	Left eye
R	5	3	4
S	7	5–6	4–5
B	7	5	5
Sp	2	5	4
P	2	3	3
T	5–6	5	5
		4.5	4 3

the eyes. As will be shown by experimental data in a later part of this paper, it is important that all of these measurements must be made on the middle of the cornea. In the case of dogs it is difficult to always touch this region.

Cats. As was found to be true for dogs, the cats also moved their eyes considerably, so that the same difficulty arose. The values for the right eye of *3 cats* lay between *5 and 6 g/mm²*.

Pigeons. The pigeons are more satisfactory for these experiments than

both dogs and cats. But the very frequent normal physiological lid-reflexes of pigeons interfere with the experiment. It is advisable, to stimulate the cornea immediately following a normal physiological lid-reflex. Naturally, one must consider in these cases that the reflex-arc may still be refractory. *The average threshold* value of 9 pigeons ranging in age from 4 weeks to 6 years was *4.2 g/mm²* (see table 3). Nos. 1, 2 and 3 were 1 to 5 years old, the remainder were young pigeons (4 to 8 weeks).

TABLE 3

PIGEON NUMBER	THRESHOLD IN G/MM²
1	4–5
2	4
3	3–4
4	5–6
5	4
6	3–4
7	5–6
8	4
9	5–6
	4 2

TABLE 4

SPECIES	THRESHOLD IN G/MM²
1 Guinea pigs	12
2 Rabbits	8–10
3 Cats	5–6
4 Dogs	4–5
5 Pigeons	4
6 Rats	1–1 5
7 Monkeys	0 5–1
8 Homo-Sapiens	0 2-0 3

Rats. In contrast to pigeons, rats seldom show any normal physiological lid-reflex. The threshold value for these animals is very low. Calculated from 10 normal adult rats, it varied between *1 and 1.5 g/mm²*.

Monkeys. Macacus rhesus are the least satisfactory subjects for these experiments because of their restlessness; only with difficulty measurements could be made on 3 monkeys; the values obtained were *0.5 to 1 g/mm²*.

Table 4 is a summary of the values obtained for the different species of all of these animals. It is not evident from these results whether or not the sensibility of the corneal nerves varies with the stage of phylogenetic

development of the species. In some instances this appears to be true, as it is the case, if we compare guinea pigs, rabbits, cats and dogs; in the cases of pigeons and rats this is not true. An investigation of a larger

TABLE 5

Rabbits

	NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	NO. 7	NO. 8	NO. 9	NO. 10
Age in months.....	3	3	3	7	9	9	9	9	18	24
Region of the eye	Threshold in g/mm²									
0	4	4	5	10	9	8	9	11	9	8
V top I	8	10	15	20	15	20	18	20	18	13
V top II	20	20	30	33	35	30	30	40	40	60
V bot. I	8	10	13	20	15	15	15	15	15	13
V bot. II	20	20	25	40	30	40	25	30	35	60
H nas. I	8	10	13	15	13	15	18	15	15	13
H nas. II	20	20	25	35	45	50	50	50	45	45
H temp. I	8	8	15	15	10	20	18	20	18	15
H temp. II	20	20	30	30	45	40	50	50	45	50
Nictitating membrane	30	30	40	45	50	50	40	50	50	50

TABLE 6

REGION OF THE EYE	AGE	
	3 months	7–24 months
	g/mm²	g/mm²
0	4–5	8–10
V top I	11	18
V top II	23	38
V bot. I	10	16
V bot. II	22	37
H nas. I	10	15
H nas. II	22	46
H temp. I	10	17
H temp. II	23	44
Membrane	33	48

number of species might give further information about this question. Without doubt, certain other factors such as thickness and hardness of the cornea-cells also may influence the results. It is interesting that the threshold value for monkeys is found to be very near to that for men as

found by M. v. Frey (2) and M. v. Frey u. H. Strughold (3) (pain). It may be added that for experimental pharmacological and pathological-physiological researches, rabbits and guinea pigs are the best, the least satisfactory are the monkeys.

As E. Marx, (4) M. v. Frey and H. Strughold (3) have already shown, the cornea and conjunctiva of man do not have the same degree of sensibility for pain in all their parts. The sensibility decreases from the center outward. If this were true also on the cornea of experimental animals above mentioned, it would be of some importance from the experimental point of view. Indeed, I found that for all species of animals examined, *the threshold values increased from the center of the cornea outward.* The

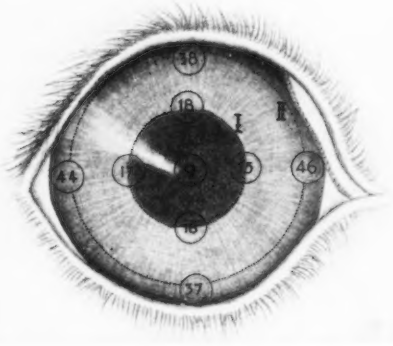

Fig. 1

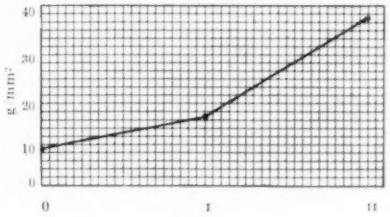

Fig. 2

Fig. 1. Diagram of the cornea of the rabbit. The numbers indicate the threshold of the corneal reflex in the different regions of the cornea in terms of g/mm².

Fig. 2. The threshold of the corneal reflex in the rabbit in terms of g/mm². The abscissa signifies the radius of the cornea. 0, center of cornea. II, border of cornea.

following detailed observations were made on *rabbits* (see tables 5 and 6 and figs. 1 and 2).

The threshold values for nine different points on the cornea were determined according to the method already described. First the center, which is designated by 0 was investigated and its threshold was found to be 9 g/mm². Furthermore, four points lying half way between center and border of the cornea on the vertical and horizontal diameter were chosen, representing zone I in figure 1. Those points in zone I and at the same time lying on the vertical diameter are indicated in the tables 5 and 6 by V. top I and V bottom I and those points in zone (I) and at the same time lying on the horizontal diameter are indicated in the figure by H. nas I.

(horizontal nasal I) and H. temp. I (horiz. temporal I). Similar marks are given to four other points lying on these diameters near the border of the cornea (zone II).

The average threshold value for the four points of this last zone (II) was found to be 41.2 and of zone I 16.5 g/mm². In relation to the threshold value of 9 g/mm² in the center, this proportion is as 4.6:1.8:1.0; that is, the threshold increases only slightly in passing from the center 0 to zone I (from 1 to 1.8) and then rapidly towards zone II (from 1.8 to 4.6). This is shown in the diagram of figure 2 in which the abscissa represents one radius of the cornea and the ordinate represents the average threshold in g/mm² for the points of zones I and II calculated as the averages of all four radii.

This corresponds with the results found for the eye of man by E. Marx, M. v. Frey u. H. Strughold, who have investigated the sensibility for pain.

It is very probable that the high sensibility of the center of the cornea (four to five times higher than near the margin) depends upon the richness in nerve endings in this part of corneal epithelium compared with the marginal zones, as is described by a number of histologists. For experimental purposes it follows from this result that it is necessary for comparison from case to case, to examine always the same point. The most practicable point is the center of the cornea, the threshold-values of which, concerning the usual laboratory animals, have been communicated n the first part of this paper and have been found little varying.

I wish to express my appreciation to Doctor Carlson for having had the privilege of working in his laboratory.

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THE ANTITRYPTIC PROPERTIES OF BLOOD SERUM

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The purpose of this paper is to set forth certain experiments on the antitryptic action of serum. No attempt will be made to discuss the bearing of these experiments on the prevailing theories of the trypsin-antitrypsin phenomenon.

Serum was used as a substrate throughout this series of experiments. The reason for this was that varying amounts of heated and unheated serum could be added without changing the concentration of the materials of the substrate. Blood was obtained from freshly killed "slaughter-house" animals. Whenever possible sheep blood was used, but occasionally it was necessary to use beef blood. After clotting, the clot was separated from the sides of the glass container and the jars placed in the refrigerator for two hours. The serum was then centrifuged and the clear cell-free supernatant liquid was used at once. The serum was used as fresh as possible in all the experiments, though we have found that serum under toluene, kept in the refrigerator, retained its antitryptic property for over a month.

The standard substrate for each flask was 50 cc. of serum and 350 cc. of pH 8 phosphate buffer. In experiments not requiring the antitryptic action of serum the substrate mixture was heated to 85°C. and cooled at once. By this means the antitryptic action was destroyed without changing the concentration of the substrate.

The dilution by the buffer prevented the serum from coagulating on heating, and also maintained a constant hydrogen ion level throughout the incubation. The total volume of 400 cc. permitted frequent sampling without material change in volume.

Sufficient toluene to cover the surface was added to each flask before incubating in a large water bath at 37.5°C. The bath was kept constantly stirred by an air current. Bacteriological examination of the flasks was carried out from time to time and plate cultures were negative.

The curve of digestion was determined by daily removal of a 40 cc. sample on which a non-protein nitrogen estimation was made by the Kjeldahl method after precipitating the protein with trichloracetic acid. All results were expressed in milligrams of nitrogen per one hundred cubic

centimeters of flask content. The trypsin used in these experiments was powdered trypsin prepared by Digestive Ferments Co.

Varying amounts of trypsin were added to the heated serum-buffer mixture, and it was found that 0.1 gram was sufficient to give a satisfactory digestion curve (chart 1). It will be noted that with increasing amounts of trypsin the digestion proceeded more rapidly and reached a higher level.

Varying amounts of trypsin were then added to flasks containing raw serum-buffer mixture. Chart 2 shows the result of this experiment. There was practically no increase in non-protein nitrogen in the flask containing 0.5 gram of trypsin. The first distinct rise occurred in the flask containing 0.75 gram. The non-protein nitrogen of the flask containing 1.0 gram of trypsin was three to four times higher than that of the flask containing 0.75 gram. The chart shows that the trypsin digested raw serum when present in sufficient quantity. However, the raw serum exerts some inhibiting effect. If all the antitrypsin of the raw serum had been used up by 0.75 gram of trypsin, then the additional 0.25 gram should have given a curve corresponding to that of 0.25 gram of trypsin acting on heated serum. By comparing chart 1 and chart 2 it is seen that this was not the case. It will be noted that the samples taken before digestion began gave higher readings with the increased amounts of trypsin. This would indicate that the trypsin used contained a large amount of split protein products.

Chart 3 illustrates the curve of digestion when 0.1 gram of trypsin was added to heated and raw serum. It will be seen from this chart that there was no action when that amount of trypsin was added to raw serum, but there was a marked effect when the same amount was added to heated serum. In some experiments there was a slight action with the raw serum, but it was considered of little account when compared with the action on heated serum.

Since 0.1 gram of trypsin had no effect on raw serum, the question arose as to whether or not the addition of raw serum to a flask which was undergoing digestion, would inhibit further action of the trypsin. Consequently 50 cc. of serum and 350 cc. of pH 8 buffer were heated to 85°C., cooled, and 0.1 gram of trypsin added. After eight hours the non-protein nitrogen had increased to 81.0 mgm. per 100 cc. At this time 50 cc. of raw serum and 350 cc. of pH 8 buffer and 0.1 gram of trypsin were added. After shaking thoroughly, the fluid was divided equally into two flasks, "3A" and "3B." Flask "3B" was heated to 85°C., cooled, and 0.1 gram of trypsin added. This flask served as a control on "3A." Chart 4, *3*, shows the result of this experiment. Chart 4, *4*, shows the result of an experiment when the raw serum was added after the flask had been digesting for 24 hours. It will be noted that the amount of non-protein nitrogen did not increase in the flasks to which the raw serum had been added. This experi-

ment shows that the introduction of a sufficient amount of raw serum causes cessation of action of trypsin which is in the process of splitting protein.

In order to further substantiate this finding, six flasks, each containing 35 cc. of raw serum and 350 cc. of water, were heated to 85°C. After cooling, 0.1 gram of trypsin was added to each. To flask 1, 15 cc. raw serum were added. All flasks were incubated in the water bath at 37.5°C. Fif-

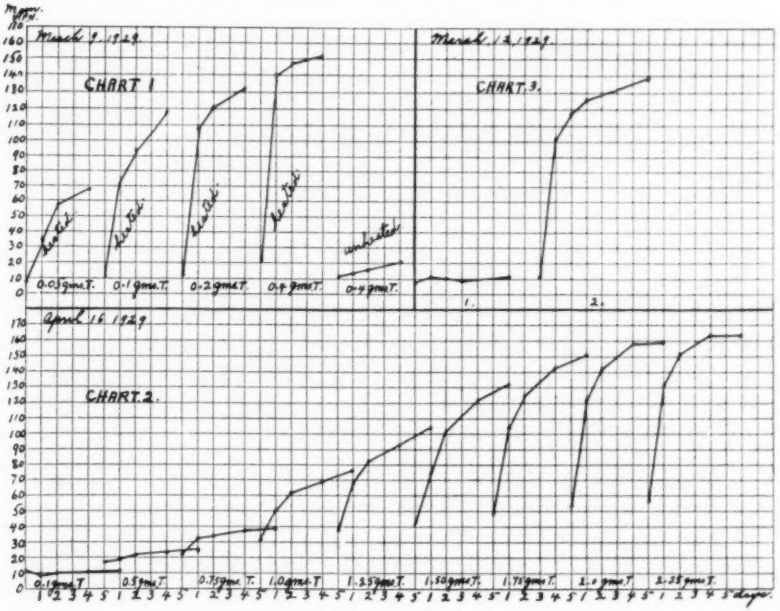

Chart 1. Milligram non-protein nitrogen in 100 cc. of fluid in flask. Each flask contains: 350 cc. pH_8 buffer, 50 cc. serum, varying amounts of trypsin.

Chart 2. Milligram non-protein nitrogen in 100 cc. of fluid in flask. Each flask contains: 350 cc. pH_8 buffer 50 cc. serum, varying amounts of trypsin.

Chart 3. Milligram non-protein nitrogen in 100 cc. of fluid in flask. Each flask contains: (1) 350 cc. pH_8 buffer + 50 cc. serum + 0.1 gram trypsin. (2) 350 cc. pH_8 buffer + 50 cc. serum (heated) + 0.1 gram trypsin.

teen cubic centimeters of raw serum were added to flask 2 in 15 minutes, to flask 3 in 30 minutes, to flask 4 in 1 hour, and to flask 5 in 2 hours. Flask 6 remained as a control. Chart 5 shows the result of this experiment. It is evident that the addition of 15 cc. of raw serum to a flask in the process of digestion caused the cessation of the tryptic action. In the case of flask 6, which had no raw serum added, the non-protein nitrogen continued to increase.

Since trypsin did not digest raw serum, but rapidly digested heated serum, it was thought that mixtures of raw and heated serums, in varying proportions, should be tested. If the raw serum did not exert an active antitryptic power its presence should not affect the digestion of heated serum. On the other hand, if it did exert an antitryptic power, even small quantities might retard or inhibit the action of the trypsin on heated serum.

A series of flasks was prepared as follows:

FLASK NUMBER	RAW SERUM	HEATED SERUM	BUFFER	TRYPSIN
	cc.	*cc.*	*cc.*	*gram*
1	50	0	350	0.1
2	40	10	350	0.1
3	30	20	350	0.1
4	25	25	350	0.1
5	20	30	350	0.1
6	10	40	350	0.1
7	0	50	350	0.1

There was no digestion in the first six flasks but a marked and rapid digestion in flask 7. This indicated that the raw serum in the mixture prevented the digestion of the heated serum. It also indicated that there was sufficient antitrypsin in 10 cc. of raw serum to control the digestion activity of 0.1 gram of trypsin.

A second series of flasks was then prepared.

FLASK NUMBER	RAW SERUM	HEATED SERUM	WATER	TRYPSIN
	cc.	*cc.*	*cc.*	*gram*
1	10	40	350	0.1
2	8	42	350	0.1
3	6	44	350	0.1
4	4	46	350	0.1
5	2	48	350	0.1
6	0	50	350	0.1

Chart 6 shows the extent of digestion in each flask. With decreasing amounts of raw serum there is an increase in the amount of digestion. Comparing the curve of flask 5 with that of flask 6 it is evident that even 2 cc. of raw serum were sufficient to limit the extent of digestion.

From other experiments it was found that the amount of antitrypsin in any serum varied to a considerable extent. In some cases 10 cc. of raw serum was sufficient to control the action of 0.1 gram of trypsin, whereas in other instances it was necessary to add as much as 25 cc. of raw serum. In order to eliminate the possibility of variations of activity of the trypsin a control flask was set up in all experiments.

SUMMARY

The action of trypsin can be controlled by raw serum. When sufficient trypsin is added to raw serum, the serum is digested. Large amounts of

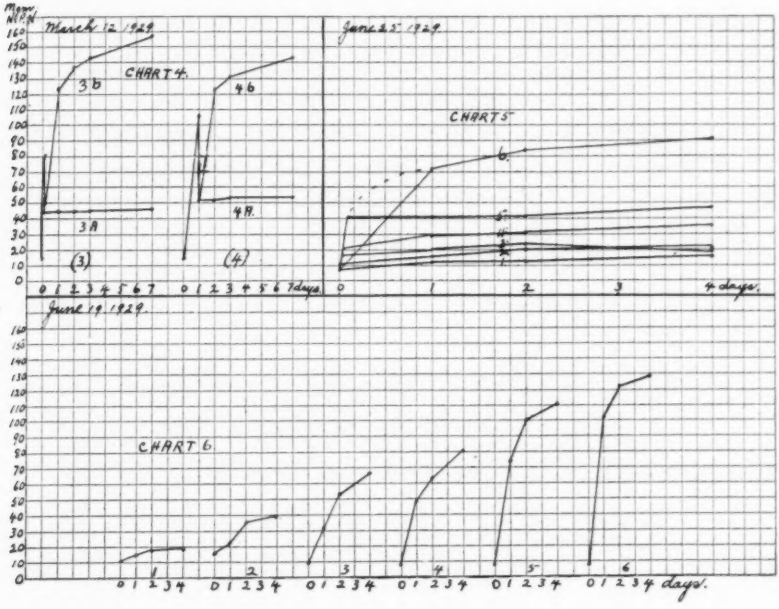

Chart 4. Milligram non-protein nitrogen in 100 cc. of fluid in flask. Each flask contains: (3) (350 cc. pH_8 buffer + 50 cc. serum) heated + 0.1 gram trypsin after 8 hours; 350 cc. pH_8 buffer + 50 cc. serum + 0.1 gram trypsin added, shaken, divided into equal quantities 3A and 3B. 3B heated, cooled and 0.1 gram trypsin added. (4) Same as 3 only 24 hours.

Chart 5. Milligram non-protein nitrogen in 100 cc. of fluid in flask. Each flask contains: 350 cc. water and 35 cc. serum heated + 0.1 gram trypsin. Fifteen cubic centimeters of raw serum were added to: (1) at once; (2) in 15 minutes; (3) in 30 minutes; (4) in 1 hour; (5) in 2 hours; (6) no serum added.

Chart 6. Milligram non-protein nitrogen in 100 cc. of fluid in flask. Each flask contains: (1) (350 cc. water + 40 cc. serum) heated + 10 cc. raw serum + 0.1 gram trypsin. (2) (350 cc. water + 42 cc. serum) heated + 8 cc. raw serum + 0.1 gram trypsin. (3) (350 cc. water + 44 cc. serum) heated + 6 cc. raw serum + 0.1 gram trypsin. (4) (350 cc. water + 46 cc. serum) heated + 4 cc. raw serum + 0.1 gram trypsin. (5) (350 cc. water + 48 cc. serum) heated + 2 cc. raw serum + 0.1 gram trypsin. (6) (350 cc. water + 50 cc. serum) heated + 0 cc. raw serum + 0.1 gram trypsin.

trypsin are required, and even with large amounts, the equilibrium between digested and undigested protein in the flask does not appear to reach as

high a level as when trypsin acts on heated serum (compare charts 1 and 2).

When trypsin is in the process of splitting heated serum the addition of sufficient raw serum immediately stops the action (charts 4 and 5). Even small amounts of raw serum (2 cc. of raw: 48 cc. of heated, chart 6) have an appreciable effect on the height of the curve of digestion.

These experiments suggest the possibility that serum exerts its inhibiting action on trypsin by means of a definite substance. However, more recent experiments, which will be published at a later data, do not substantiate this theory.

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